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PATENT ABSTRACTS OF JAPAN

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(54) POLYPEPTIDE-CONTAINING SUBSTRATE

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a substrate providing high adhesiveness and good breeding condition to a cell, and enabling the cell to be efficiently cultured by a serum- free medium.
SOLUTION: The polypeptide-containing substrate contains a polypeptide (P) having at least one minimum amino acid sequence exhibiting a cell adhesion signal in one molecule, and has (A) at least one functional group selected from the group consisting of (A1) a secondary amino group, (A2) a tertiary amino group, (A3) an ammonio group, (A4) a phosphatidyl group and (A5) a lysophosphatidyl group, and/or (B) at least one structure selected from (B1) sugar and (B2) a steroid ring.

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CLAIMS

[Claim(s)]
[Claim 1] It comes to contain the polypeptide (P) which has the minimum amino acid sequence showing a cell adhesion signal in [at least one] 1 molecule. And the 2nd class amino group (A1), the 3rd class amino group (A2), an ammonio radical (A3), At least one functional group chosen from the group which consists of a phosphatidyl radical (A4) and a RIZOHOSUFACHIJIRU radical (A5) (A), The polypeptide content base material characterized by coming to have at least one structure (B) chosen as a list from/or sugar (B1), and a steroid ring (B-2).
[Claim 2] The polypeptide content base material according to claim 1 with which all or a part of a polypeptide (P), functional group (A), and/or structure (B) come to exist in a base material front face.
[Claim 3] The polypeptide content base material according to claim 1 or 2 whose content (mug/cm2) of a polypeptide (P) is 100 or less [0.1 or more / per two] the surface area of 1cm of a base material.
[Claim 4] The polypeptide content base material according to claim 1 to 3 whose number showing the cell adhesion signal of a polypeptide (P) of the minimum amino acid sequences is 3-50 in (P)1 molecule.
[Claim 5] The minimum amino acid sequence showing the cell adhesion signal of a polypeptide (P) An Arg Gly Asp array, a Leu Asp Val array, an Arg Glu Asp Val array (1), A TyrIle Gly Ser Arg array (2), a Pro Asp Ser Gly Arg array (3), An Arg Tyr Val Val Leu Pro Arg array (4), A Leu Gly Thr Ile Pro Gly array (5), an Arg Asn IleAla Glu Ile Ile Lys Asp Ile array (6), An Ile Lys Val Ala Val array (7), a Leu Arg Glu array, The polypeptide content base material according to claim 1 to 4 which are at least one sort of arrays chosen from the group which consists of an Asp Gly Glu Ala array (8) and a His Ala Val array.
[Claim 6] The polypeptide content base material according to claim 1 to 5 whose content of a functional group (A) and/or structure (B) is 1x1013 to 1x1020 per two the surface area of 1cm of a base material.
[Claim 7] A functional group (A) A dimethylamino radical, a trimethylammonio radical, a diethylamino radical, In a diethyl methyl ammonio radical and/or a guanidino radical, and a list,/or the polycondensation object of a dicyandiamide and formalin, The polycondensation object of a dicyandiamide and diethylenetriamine, the addition polymerization object of epichlorohydrin and dimethylamine, The 2nd class amino group contained in at least one polymer chosen from the group which consists of a dimethyl diaryl AMIMMONIUMU chloride polymerization object and a copolymerization object of a diaryl amine hydrochloride and a sulfur dioxide, The polypeptide content base material according to claim 1 to 6 which is the 3rd class amino group, an ammonio radical, an imino group, an amidino group and/, or a GUAJINO radical.
[Claim 8] The polypeptide content base material according to claim 1 to 7 whose or more 50 100 or less and consistency (g/cm3) volume mean particle diameter (micrometer) is or more 1.02 1.04 or less particle for a base material.
[Claim 9] Claims 1-9 which come to contain the synthetic macromolecule which becomes considering styrene and a polyfunctional monomer as an indispensable configuration monomer are the polypeptide content base materials of a publication either.
[Claim 10] The process of the animal cell characterized by cultivating an animal cell in a serum free medium using a polypeptide content base material according to claim 1 to 9.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[Field of the Invention]
[0001]
This invention relates to a wound cladding material. It is related with the wound cladding material which has a cellular adhesiveness polypeptide in more detail.
[Background of the Invention]
[0002]
Conventionally, the wound cladding material (patent reference 1) into which the collagen of the natural origin was processed a sheet and in the shape of sponge, the fibroblast content collagen sheet (patent reference 2) which cultivates the fibroblast of the skin origin on a collagen sheet or in a collagen sheet, and is obtained are known as a wound cladding material which has a cellular adhesiveness polypeptide.
[0003]
[Patent reference 1] JP,7-59812,A
[Patent reference 2] JP,9-47502,A
[Description of the Invention]
[Problem(s) to be Solved by the Invention]
[0004]
Since a main constituent was protein, there were problems, such as infection by the microorganism which has a bad influence on the body, the microorganism origin matter, etc. mixing into a wound cladding material, and it will collapse by the exudate from a wound and the gestalt of a wound cladding material will be lost, and the wound cladding material which has the conventional cellular adhesiveness polypeptide had the trouble of being unable to perform a stick substitute, when applying a wound cladding material to a wound side. That is, even if the purpose of this invention has very low mixing of the microorganism which has a bad influence on the body, the microorganism origin matter, etc. and has contact with an exudate etc., it is offering the wound cladding material with which the gestalt of a wound cladding material is maintained.
[Means for Solving the Problem]
[0005]
As a result of coming research in piles wholeheartedly, by using a specific peptide and specific covering material, this invention person found out attaining the above-mentioned purpose, and reached this invention. That is, the description of the wound cladding material of this invention makes a summary the point that the content of endotoxin consists of a cellular adhesiveness polypeptide (P) which are under 0.15EU/mg, and covering material (N) based on the weight of a cellular adhesiveness polypeptide (P).
[Effect of the Invention]
[0006]
The wound cladding material of this invention has very few endotoxin contents, and a cell can be pasted up very efficiently on a wound cladding material. Furthermore, if the wound cladding material of this invention is applied to a wound side, it will change into a very good playback condition, without a wound side infecting and conglutinating. Therefore, the wound cladding material of this invention has little danger of mixing, such as a microorganism and microorganism origin matter, and a stick substitute of a wound cladding material is easy for it, and it can promote recovery of a wound.
[Best Mode of Carrying Out the Invention]
[0007]
The cellular adhesiveness polypeptide (P) includes the minimum amino acid sequence (X) showing a cell adhesion signal. As the minimum amino acid sequence (X) showing a cell adhesion signal, what is indicated in "pathophysiology, volume [9th] No. 7, 527-535 pages, 1990", and "an Osaka prefectural mother-and-child medical center magazine, volume [8th] No. 1, 58-66 pages and 1992" is used, for example.
[0008]
In these minimum amino acid sequences (X), an Arg Gly Asp array, A Leu Asp Val array, an Arg Glu Asp Val

array (1), A Tyr Ile Gly Ser Arg array (2), a Pro Asp Ser Gly Arg array (3), An Arg Tyr Val Val Leu Pro Arg array (4), A Leu Gly Thr Ile Pro Gly array (5), an Arg Asn Ile Ala Glu Ile Ile Lys Asp Ile array (6), An Ile Lys Val Ala Val array (7), a Leu Arg Glu array, An Asp Gly Glu Ala array (8), a Gly Val Lys Gly Asp Lys Gly Asn Pro Gly Trp Pro Gly Ala Pro array (9), A Gly Glu Phe Tyr Phe Asp Leu Arg Leu Lys Gly Asp Lys array (10), A His Ala Val array and a Tyr Lys Leu Asn Val Asn Asp Ser array (11) are desirable. From a viewpoint of cellular adhesiveness, it is an Arg Gly Asp array, a Tyr Ile Gly Ser Arg array (2), and an Ile Lys Val Ala Val array (7) still more preferably, and is an Arg Gly Asp array especially preferably. in addition, an amino acid sequence -- a three-character notation -- expressing -- () -- the array number corresponding to an array table is indicated inside (it is the same hereafter.).

[0009]

Although a cellular adhesiveness polypeptide (P) should just have said minimum amino acid sequence (X) in [at least one] 1 molecule What it has in [two or more] 1 molecule from a viewpoint of cellular adhesiveness is desirable. What it has in [50 or less] 1 molecule is desirable, what it has three or more pieces is still more desirable, and especially the thing that it has five or more pieces is desirable, and especially the thing that it has 20 or less pieces is [what it has 30 or less pieces is still more desirable, and] desirable. Moreover, two or more sorts of arrays may be included in a monad.

[0010]

From a viewpoint of cellular adhesiveness, 3,000,000 or less are desirable still more desirable, and the number average molecular weight (Mn) of a cellular adhesiveness polypeptide (P) is 300,000 or less especially preferably 1,000,000 or less, and 300 or more are 3,000 or more especially preferably 1,000 or more desirable still more preferably. in addition, Mn of a cellular adhesiveness polypeptide (P) -- SDS-PAGE (SDS polyacrylamide gel electrophoresis) -- it is law, a cellular adhesiveness polypeptide (P) is separated, and it asks by comparing migration distance with the standard substance (it is the same hereafter.).

[0011]

As a cellular adhesiveness polypeptide (P), although a cellular adhesiveness natural polypeptide (P1), a cellular adhesiveness artificial polypeptide (P2), etc. can be used, a cellular adhesiveness artificial polypeptide (P2) is desirable at the point that the number of the minimum amino acid sequence (X) showing a cell adhesion signal and heat-resistant amino acid sequences (Y), arrangement, etc. can be designed freely, cellular adhesiveness is raised or sterilization by heat-treatment and decomposition of endotoxin can be made easy.

[0012]

As a cellular adhesiveness natural polypeptide (P1) the sugar protein (for example, a laminin and entactin (NAIDOJIEN) --) which exists in basement membrane TENEISHIN, AGURIN, osteonectin, osteocalcin, osteopontin, FIBURUIN, a fibrinogen, vitronectin, support phosphorus, BAMIN, a thrombospondin, etc., proteoglycan (BIGURIKAN for example, AGURIKAN and a pearl can --) Decorin, FIBUROMOJURIN, bar SHIKAN, DEYURIN, a neuro-can, BUREBIKAN, a roux mandarin orange, a cel glycine, Cindy Quan, CD44, beta glycan, TRON BOMODEYURIN, GURIPIKAN, SEREBURO glycan, NG2 proteoglycan, etc., The sugar proteins (for example, integrin, an integrin super family, cadherin, a cadherin super family, etc.) which exist in a cell membrane, the matter (for example, OKURU DIN etc.) about a tight junction, etc. are mentioned.

[0013]

As a cellular adhesiveness artificial polypeptide (P2) For example, the polypeptide which consists of a Tyr Ile Gly Ser Arg array (2), The polypeptide which consists of an Ile Lys Val Ala Val array (7), The polypeptide which consists of an Arg Gly Asp Ser array (14), The polypeptide which consists of a Gly Arg Gly Asp Ser array (15), The polypeptide which consists of a Gly Arg Gly Asp Ser Pro array (16), The polypeptide which consists of an Arg Gly Asp Ser Pro Ala Ser Ser Lys Pro array (17), The polypeptide which consists of an Ala Val ThrGly Arg Gly Asp Ser Pro Ala Ser Ala array (18), The polypeptide which consists of a Pro Gly Ala Ser Ile Lys Val Ala Val Ser Ala Gly Pro Ser array (19), The polypeptide which consists of a Cys Ser Arg Ala Arg Lys Gln Ala Ala Ser Ile Lys Val Ala Val Ser Ala Asp Arg array (20), The polypeptide which consists of a Val Cys Glu Pro Gly Tyr Ile Gly Ser Arg Cys Asp array (21), the polymer which consists of these polypeptides of a kind of at least can be illustrated. Besides these, as a polymer, for example, the polymer which consists of four (Arg Gly Asp Ser) arrays (22), The polymer which consists of eight arrays (23), (Arg Gly Asp Ser) The polymer which consists of 16 arrays (24), (Arg Gly Asp Ser) The polymer which consists of eight arrays (25), (Gly Arg Gly Asp Ser) The polymer which consists of eight arrays (26), (Gly Arg Gly Asp Ser Pro) The polymer which consists of four arrays (27), (Arg Gly Asp Ser Pro Ala Ser Ser Lys Pro) The polymer which consists of four arrays (28), (Ala Val Thr Gly Arg Gly Asp Ser Pro Ala Ser Ala) The polymer which consists of four arrays (29), (Pro Gly Ala Ser IleLys Val Ala Val Ser Ala Gly Pro Ser) The polymer which consists of four arrays (30), (Cys Ser Arg Ala Arg Lys Gln Ala Ala Ser Ile Lys Val Ala Val Ser Ala Asp Arg) Or the polymer which consists of four (Val Cys Glu Pro Gly Tyr Ile Gly Ser Arg Cys Asp) arrays (31) is mentioned. As for the polymerization degree (repeat unit number) of this polymer, two or more are desirable, three or more are still more desirable, and four especially or more are desirable, and 50 or less are desirable, 30 or less are still more desirable, 20 especially or less are desirable, and 16 or less are

the most desirable than the viewpoint of cellular adhesiveness.
[0014]
As for a cellular adhesiveness artificial polypeptide (P2), it is desirable to include heat-resistant amino acid sequences (Y) other than the minimum amino acid sequence (X) which expresses a cell adhesion signal further. As a heat-resistant amino acid sequence (Y), a Gly Ala Gly Ala Gly Ser array (12), A Gly Val Gly Val Pro array (13), a Gly Pro Pro array, A Gly Ala Gln Gly Pro Ala Gly Pro Gly array (32), A Gly Ala Pro Gly Ala Pro Gly Ser Gln Gly Ala Pro Gly Leu Gln array (33), A Gly Ala Pro Gly Thr Pro Gly Pro Gln Gly Leu Pro Gly Ser Pro array (34), A Gly Ala array, a Gly Ala Gly Ala Gly Tyr (35) array, A Gly Ala Gly Val Gly Tyr array (36), a Gly Ala Gly Tyr Gly Val array (37), An Asp Gly Gly 12 (Ala) Gly Gly Ala array (38), a Gly Val Pro Gly Val array (39), Gly and Ala, a Gly Gly Ala array, etc. are mentioned. If these heat-resistant amino acid sequences (Y) are included, the stability over heat will increase further and it will become easy to carry out heat sterilization of a cellular adhesiveness polypeptide or the cellular adhesiveness polypeptide content base material with an autoclave etc. Since the thermal resistance which was excellent among these thermal-resistance amino acid sequences (Y) is obtained, a Gly Ala Gly Ala Gly Ser array (12), a Gly Val Gly Val Pro array (13), and a Gly Pro Pro array are Gly Ala Gly Ala Gly Ser arrays (12) desirable still more preferably.
[0015]
As for a heat-resistant amino acid sequence (Y), it is desirable that congener or (Y) of a different kind has repeated in order to raise the stability over heat further. 2-100 pieces are desirable still more desirable, and 3-50 pieces, the polymerization degree (repeat unit number) of a heat-resistant amino acid sequence (Y) is 4-30 pieces especially preferably, and is 4-20 pieces most preferably.
When it includes a heat-resistant amino acid sequence (Y), although (Y) should just be contained in which location of a cellular adhesiveness artificial polypeptide (P2), as for (Y) and an amino acid sequence (X), it is desirable [Y] that the polymer of (Y) and (X) are located by turns from a viewpoint of the ease of taking of the beta turn structure of a cellular adhesiveness artificial polypeptide (P2).
[0016]
When a cellular adhesiveness artificial polypeptide (P2) comes to have a heat-resistant amino acid sequence (Y), the content of a heat-resistant amino acid sequence (Y) From a viewpoint of stability over heat, in 1 molecule of a cellular adhesiveness artificial polypeptide (P2) 1,000 or less things which ten or more things [30 or more] which it has three or more pieces have especially preferably, and have 10,000 or less pieces desirable still more preferably have 3,000 or less pieces preferably especially desirable still more preferably.
[0017]
The following polypeptide the cellular adhesiveness artificial polypeptide (P2) is indicated to be by a ****** 3-No. 502935 official report and Handbook of Biodegradable Polymers, Harwood Academic Publishers, and Amsterdam as a thing which comes to have a heat-resistant amino acid sequence (Y), for example is mentioned. Namely, Mn about 100,000 polypeptide which has nine (Gly Ala Gly Ala Gly Ser) arrays (40) and about 13 Arg Gly Asp arrays at a time, respectively (SLPF), Mn about 100,000 polypeptide which has nine arrays (40) and about 13 Tyr Ile Gly Ser Arg arrays (2) at a time, respectively, (Gly Ala Gly Ala Gly Ser) Mn about 100,000 polypeptide which has nine arrays (40) and about 13 Ile Lys Val Ala Val arrays (7) at a time, respectively, (Gly Ala Gly Ala Gly Ser) Mn about 100,000 polypeptide which has eight arrays (41), and about 12 12 (Gly Ala Gly Ala Gly Ser) arrays (42) and Arg Gly Asp arrays at a time, respectively, (Gly Val Gly Val Pro) And (Gly Ala Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly ProPro) Mn about 50,000 polypeptide etc. which has 2 (43) arrays and about six Arg Gly Asp arrays at a time, respectively is mentioned.
[0018]
As a cellular adhesiveness artificial polypeptide (P2) which comes to have a heat-resistant amino acid sequence (Y) above else, the polypeptide which has the structure the minimum amino acid sequence (X) shown in the following (1) - (3) and a heat-resistant amino acid sequence (Y) come to carry out a chemical bond by turns can be used.
(1) When the minimum amino acid sequence (X) is an Arg Gly Asp array (x1)
The polypeptide which has 5 of (x1), and 4 of a Ser Pro Ala Gly Gly3(Ala Gly Ala Gly Ser Gly) Ala Ser Thr Gly array (44) and (y1) (45), The polypeptide which has 10 of (x1), and 9 of (y1) (46), The polypeptide which has 15 of (x1), and 14 of (y1) (47), Four pieces and Ser Pro Ala 2 (Gly Val Pro Gly Val) Gly Gly3(Gly Ala Gly Ala Gly Ser) Ala Ser Thr Gly of (x1) Three of an array (48) and (y2) The polypeptide which has 8 of the polypeptide (49) which it has, and (x1), and 7 of (y2) (50), The polypeptide which has 12 of (x1), and 11 of (y2) (51), Five pieces and Ser Pro Ala Ala Ser Asp Gly Gly 12 (Ala) Gly Gly Ala AlaSer Thr Gly of (x1) The polypeptide which has four of an array (52) and (y3) (53), The polypeptide which has 10 of (x1), and 9 of (y3) (54), The polypeptide which has 15 of (x1), and 14 of (y3) (55), the polypeptide (58) which has 10 of the polypeptide (57) which has 5 of (x1), and 4 of 13 (Gly Ala) arrays (y4) (56), and (x1), and 9 of (y4) — and (x1) the polypeptide (59) which has 15 pieces and 14 of (y4).
[0019]

(2) When the minimum amino acid sequences (X) are a Tyr Ile Gly Ser Arg array (2) and (x2)
The polypeptide which has 5 of (x2), and 4 of a Ser Pro Ala Gly Gly3(Ala Gly Ala Gly Ser Gly) Ala Ser Thr Gly array (44) and (y1) (60), The polypeptide which has 10 of (x2), and 9 of (y1) (61), The polypeptide which has 15 of (x2), and 14 of (y1) (62), Four pieces and Ser Pro Ala 2 (Gly Val Pro Gly Val) Gly Gly3(Gly Ala Gly Ala Gly Ser) Ala Ser Thr Gly of (x2) Three of an array (48) and (y2) The polypeptide which has 8 of the polypeptide (63) which it has, and (x2), and 7 of (y2) (64), The polypeptide which has 12 of (x2), and 11 of (y2) (65), Five pieces and Ser Pro Ala Ala Ser Asp Gly Gly 12 (Ala) Gly Gly Ala AlaSer Thr Gly of (x2) The polypeptide which has four of an array (52) and (y3) (66), The polypeptide which has 10 of (x2), and 9 of (y3) (67), The polypeptide which has 15 of (x2), and 14 of (y3) (68), The polypeptide (70) which has 10 of the polypeptide (69) which has 5 of (x2), and 4 of 13 (Gly Ala) arrays (y4) (56), and (x2), and 9 of (y4), the polypeptide which has 15 of (x2), and 14 of (y4) (71).

[0020]

(3) When the minimum amino acid sequences (X) are an Ile Lys Val Ala Val array (7) and (x3)
The polypeptide which has 5 of (x3), and 4 of a Ser Pro Ala Gly Gly3(Ala Gly Ala Gly Ser Gly) Ala Ser Thr Gly array (44) and (y1) (72), The polypeptide which has 10 of (x3), and 9 of (y1) (73), The polypeptide which has 15 of (x3), and 14 of (y1) (74), Four pieces and Ser Pro Ala 2 (Gly Val Pro Gly Val) Gly Gly3(Gly Ala Gly Ala Gly Ser) Ala Ser Thr Gly of (x3) Three of an array (48) and (y2) The polypeptide which has 8 of the polypeptide (75) which it has, and (x3), and 7 of (y2) (76), The polypeptide which has 12 of (x3), and 11 of (y2) (77), Five pieces and Ser Pro Ala Ala Ser Asp Gly Gly 12 (Ala) Gly Gly Ala AlaSer Thr Gly of (x3) The polypeptide which has four of an array (52) and (y3) (78), The polypeptide which has 10 of (x3), and 9 of (y3) (79), The polypeptide which has 15 of (x3), and 14 of (y3) (80), the polypeptide (82) which has 10 of the polypeptide (81) which has 5 of (x3), and 4 of 13 (Gly Ala) arrays (y4) (21), and (x3), and 9 of (y4) — and (x3) the polypeptide (83) which has 15 pieces and 14 of (y4).

[0021]

As a cellular adhesiveness artificial polypeptide (P2) which can come to hand from a commercial scene The polypeptide which will consist of a RGDS[Arg Gly Asp Ser array (14), for example if a trade name is indicated, Mn 400 [about],] by the peptide lab company, the polypeptide that consists of a GRGDS[Gly Arg Gly Asp Ser array (15), It has respectively Mn 500 [about],] by the peptide lab company, a PURONE cutin F[Arg Gly Asp array, and nine (Gly Ala Gly Ala Gly Ser) arrays [about 13] (40). The polypeptide, Mn 100,000 [about] which are manufactured with transgenics Escherichia coli,] by Sanyo Chemical Industries, Ltd., the thing which the PURONE cutin F plus [PURONE cutin F was made to react with JIMERU aminoethyl chloride, and was made into water solubility, It has respectively a] [by Sanyo Chemical Industries, Ltd.], and PURONE cutin L[Ile Lys Val Ala Val array (7), and nine (Gly Ala Gly Ala Gly Ser) arrays [about 13] (40). The polypeptide manufactured with transgenics Escherichia coli, Mn 100,000 [about],] by Sanyo Chemical Industries, Ltd., etc. are mentioned.

[0022]

A cellular adhesiveness artificial polypeptide (P2) is manufactured artificially, and can be easily manufactured by organic synthesis methods (enzymatic process, a solid phase synthesis method, liquid phase synthesis method, etc.), gene recombination, etc. About an organic synthesis method, the biochemistry experiment lecture 1, the proteinic chemistry IV (July 1, 1981, edited by Japanese Biochemical Society, Tokyo Kagaku Dojin Issue) or the ******** experiment lecture 2, the approach indicated by proteinic chemistry (below) (May 20, Showa 62, edited by Japanese Biochemical Society, Tokyo Kagaku Dojin Issue) are applicable, for example. About gene recombination, the approach indicated by the ****** No. 502935 [three to] official report is applicable, for example. In addition, since the impurity of the recombination microorganism origin may be included when based on gene recombination, it is 95 % of the weight or more for the affinity purification using an anti-polypeptide antibody etc. to refine, and to carry out purity of a polypeptide to 80% of the weight or more especially preferably 90% of the weight or more desirable still more preferably. The viewpoint that the amino acid sequence of a cellular adhesiveness polypeptide can be designed and manufactured easily to gene recombination is [among these] desirable.

[0023]

As a cellular adhesiveness artificial polypeptide by the organic synthesis method For example, the polypeptide which consists of an Arg Gly Asp Ser array (14) (Mn 400 [about]), The polypeptide which consists of a Gly Arg Gly Asp Ser array (15) (Mn 500 [about]), The polypeptide which consists of a Gly Arg Gly Asp Ser Pro array (16) (Mn 600 [about]), Or polypeptides, such as a polypeptide (Mn 1000 [about]) which consists of an Arg Gly Asp Ser Pro Ala Ser Ser Lys Pro array (17), other polymers, etc. are used. The polymer which consists of four (Arg Gly Asp Ser) arrays (22) as the above-mentioned polymer, for example (Mn 1700 [about]), The polymer which consists of eight arrays (23) (Mn 3000 [about]), (Arg Gly Asp Ser) The polymer which consists of 16 arrays (24) (Mn 7000 [about]), (Arg Gly Asp Ser) The polymer which consists of 8 (25) (Mn 4000 [about]), (Gly Arg Gly Asp Ser) (Gly Arg Gly Asp Ser Pro) The polymer (Mn 5000 [about]) which consists of 8 (26), or the polymer (Mn 4000 [about]) which consists of 4 (Arg Gly Asp Ser Pro Ala Ser Ser Lys Pro) (27) is mentioned.

the polymerization degree (repeat unit number) of these polymers — 2–50 — desirable — further — desirable — 3–30 — especially — desirable — 4–20 — it is 4–16 most preferably.

[0024]

As a cellular adhesiveness artificial polypeptide by gene recombination For example, Mn about 100,000 polypeptide which has nine (Gly Ala Gly Ala Gly Ser) arrays (40) and about 13 Arg Gly Asp arrays at a time, respectively (SLPF), Mn about 100,000 polypeptide which has nine arrays (40) and about 13 Tyr Ile Gly Ser Arg arrays (2) at a time, respectively, (Gly Ala Gly Ala Gly Ser) Mn about 100,000 polypeptide which has nine arrays (40) and about 13 Ile Lys Val Ala Val arrays (7) at a time, respectively, (Gly Ala Gly Ala Gly Ser) Mn about 100,000 polypeptide which has eight arrays (41), and about 12 12 (Gly Ala Gly Ala Gly Ser) arrays (42) and Arg Gly Asp arrays at a time, respectively, (Gly Val Gly Val Pro) And (Gly Ala Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly ProPro) Mn about 50,000 polypeptide etc. which has 2 (43) arrays and about six Arg Gly Asp arrays at a time, respectively is mentioned.

[0025]

As for a cellular adhesiveness polypeptide (P), it is desirable that can hold many cells on a wound cladding material, and the recovery period is embellished with the compound (AM) containing the amino group and/or an ammonio radical from a viewpoint that it can be shortened more.

[0026]

As a compound (AM) containing the amino group and/or an ammonio radical, the 4th class ghosts of these etc. can be used for the polymer list of the halogenide, amino-group content monomer, and amino-group content monomer which have polyamine, amino alcohol, and an amino group.

As polyamine, the polyamine (carbon numbers 2–56) which has the at least one 1st class amino group or the 2nd class amino group is used, and aliphatic series polyamine, alicyclic polyamine, heterocycle type polyamine, aromatic series polyamine, etc. are used.

[0027]

as aliphatic series polyamine — alkylene diamine (ethylenediamine —) Propylenediamine, trimethylene diamine, a tetramethylenediamine, a hexamethylenediamine, etc., the polyalkylene polyamine (diethylenetriamine —) whose carbon numbers of an alkylene group are 2–6 Iminobis propylamine, triethylenetetramine, tetraethylenepentamine, pentaethylenehexamine, etc., and these alkyl (carbon numbers 1–18) substitution products (dimethylamino propylamine —) Diethylamino propylamine, dipropylamino propylamine, methylethylamino propylamine, trimethyl hexamethylenediamine, N, and N-dioctadecyl ethylenediamine, trio KUTADE sill ethylenediamine, methyliminobispropylamine, etc. — etc. — it is mentioned.

[0028]

As alicyclic polyamine, a 1, 3-diamino cyclohexane, 1, 3-screw (methylamino) cyclohexane, 1, 3-screw (dihydroxy amino) cyclohexane, isophorone diamine, menthonaphtene diamine and 4, and 4'-methylene JISHIKURO hexanediamine etc. is mentioned.

As heterocycle type polyamine, piperazine, N-methyl piperazine, N-aminoethyl piperazine and 1, and 4-diamino ethyl piperazine etc. is mentioned.

As aromatic series polyamine, a phenylenediamine, N, and N'-dimethyl phenylenediamine, N and N, - trimethyl phenylenediamine, diphenylmethane diamine and 2, 6-diamino pyridine, tolylenediamine, diethyl tolylenediamine, and N'4, 4'-screw (methylamino) diphenylmethane, 1-methyl-2-methylamino-4-aminobenzene, etc. are mentioned.

[0029]

As amino alcohol, the amino alcohol of carbon numbers 2–58 etc. is used. The alkanolamine [monoethanolamine of carbon numbers 2–10, diethanolamine, Triethanolamine, monoisopropanolamine, a mono-butanol amine, Triethanolamine, tripropanolamine, tributanolamine, N and N-screw (hydroxyethyl) ethylenediamine and N and N, N',], these alkyl (carbon numbers 1–18) substitution product [N,N-dimethylethanolamine, such as N'-tetrakis (hydroxyethyl) ethylenediamine, N,N-diethylethanolamine, N-ethyl diethanolamine, N-octadecyl diethanolamine, N, and N-diethyl - N', N'-screw (hydroxyethyl) ethylenediamine, N — N - dioctadecyl - N — ' — N — ' - a screw (hydroxyethyl) — ethylenediamine — and — N — N — N — ' - a trio — KUTADESHIRU - N — ' - hydroxyethyl — ethylenediamine — etc. —] — etc. — mentioning — having .

[0030]

As a halogenide which has an amino group, the halogens (chlorine, bromine, etc.) ghost of the alkylamine of carbon numbers 2–17 etc. is used, and aminoethyl chloride, N-methylamino pro PIRUKURORIDO, dimethylamino ethyl chloride, diethylamino ethyl chloride, a dibenzylamino ethyl bromide, dimethylaminopropyl bromide, diethylamino propyl chloride, dibenzylamino propyl chloride, etc. are mentioned.

[0031]

As an amino-group content monomer, the amino-group content vinyl compound of carbon numbers 5–21, ethyleneimine, the amino acid of carbon numbers 2–20, etc. are used.

As an amino-group content vinyl compound, amino-group content (meta) acrylate, amino-group content (meta)

acrylamide, an amino-group content aromatic series vinyl hydrocarbon, the amino-group content allyl compound ether, etc. are used. In addition, acrylamide (meta) means acrylamide and/or methacrylamide.
[0032]
As amino-group content (meta) acrylate Aminoethyl (meta) acrylate, N-methylamino ethyl (meta) acrylate, N and N-dimethylaminoethyl (meta) acrylate, N, and N-diethylamino propyl (meta) acrylate, N and N-dipropyl aminoethyl (meta) acrylate, N-benzyl-N-methylamino ethyl (meta) acrylate, N and N-dibenzyl aminoethyl (meta) acrylate, N, and N-dibenzyl aminopropyl (meta) acrylate, morpholino ethyl (meta) acrylate, N-methyl PIPECHIJINO ethyl (meta) acrylate, etc. are mentioned.
[0033]
As amino-group content (meta) acrylamide, aminoethyl acrylamide, N-methylamino propylure krill amide, N, and N-dimethylaminoethyl (meta) acrylamide, N, and N-diethylamino propyl (meta) acrylamide, N, and N-dipropyl aminoethyl (meta) acrylamide, N-benzyl-N-methylamino ethyl (meta) acrylamide, morpholino ethyl (meta) acrylamide, N-methyl PIPECHIJINO ethyl (meta) acrylamide, etc. are mentioned.
[0034]
As an amino-group content aromatic series vinyl hydrocarbon, aminoethyl styrene, N-methylamino ethyl styrene, N, and N-dimethylamino styrene, N, and N-dipropylamino styrene, N-benzyl-N-methylamino styrene, etc. are mentioned.
As the amino-group content allyl compound ether, aminoethyl allyl compound ether, N-methylamino ethyl allyl compound ether, N, and N-dimethylaminoethyl allyl compound ether and N, and N-diethylaminoethyl allyl compound ether etc. is mentioned.
[0035]
As amino acid, an arginine, a histidine, an isoleucine, a leucine, a methionine, a phenylalanine, threonine, a tryptophan, a thyrosin, a valine, an alanine, an asparagine, an aspartic acid, a glutamine, glutamic acid, a proline, a cysteine, a lysine, a serine, a glycine, 3-aminopropionic acid, 8-amino bitter taste tongue acid, 20-amino eicosanoic acid, etc. are mentioned.
[0036]
As a polymer of an amino-group content monomer, vinyl polymer, polyethyleneimine, a polypeptide (a cellular adhesiveness polypeptide (P) is not included.), etc. which consist of an amino-group content vinyl compound are mentioned. 500 or more weight average molecular weight of the polymer of an amino-group content monomer is 2,000 or more especially preferably 1,000 or more desirable still more preferably, and 1,000,000 or less are 500,000 or less especially preferably 800,000 or less desirable still more preferably. In addition, weight average molecular weight can be measured with gel permeation chromatography (GPC). In addition, as a reference material, the polystyrene standard (TOSOH make) of molecular weight 420-20,600,000 can be used, for example.
[0037]
As the 4th class ghosts of these, that which formed these amino groups into 4 class by the 4th class-ized agents (methyl chloride, ethyl chloride, benzyl chloride, dimethyl carbonic acid, a dimethyl sulfate, ethylene oxide, etc.) is mentioned.
[0038]
As an approach of embellishing with the compound (AM) containing the amino group and/or an ammonio radical For example, the approach to which the compound (AM) containing the amino group and/or an ammonio radical and the cellular adhesiveness polypeptide before qualification are made to react, The approach of making the cellular adhesiveness polypeptide before qualification carrying out physical adsorption of the compound (AM) containing the amino group and/or an ammonio radical to a list etc. is applicable. The approach The same chemical association and/or same physical adsorption as (1) - (3) can be used among the joint approaches of of the cellular adhesiveness polypeptide (P) and covering material (N) which are mentioned later, and the same is said also of desirable chemical association and/or desirable physical adsorption.
[0039]
100,000 or less [per cellular adhesiveness (polypeptide P) 1 molecule] are desirable still more desirable, and the average number (individual) of the amino group of the cellular adhesiveness polypeptide embellished with the compound (AM) containing the amino group and/or an ammonio radical is 1,000 or less especially preferably 10,000 or less, and 0.001 or more are 0.1 or more especially preferably 0.01 or more desirable still more preferably.
[0040]
the approach that the average number of the above-mentioned amino group is well-known -- a quantum -- it can do -- as a well-known approach -- for example, trinitro benzenesulfonic acid (TNBS) -- law [the proteinic chemistry IV (the Tokyo Kagaku Dojin issue, 1981)] etc., all amine ******** [JIS K 7237-1986 and ASTM D2074-66 grade] by the hydrochloric acid and indicator titrimetric methods (bromphenol blue etc.), etc. are mentioned.
specifically, the number of the amino group changes the concentration per unit volume in the compound (AM)

containing the known amino group and/or a known ammonio radical — making — TNBS — it measures by law and a calibration curve (the number of the amino group and graph of an absorbance) is produced. moreover, the cellular adhesiveness polypeptide before qualification (P) — TNBS — by law, it measures and the obtained absorbance is converted into the number of the amino group using a calibration curve. the cellular adhesiveness polypeptide after embellishing in coincidence — TNBS — by law, it measures and the obtained absorbance is converted into the number of the amino group using a calibration curve. The difference of the number of the amino group before and behind these qualification is computed, and it considers as the average number of the amino group embellished with **(ing) by the molecularity of a cellular adhesiveness polypeptide (P) which used the value for measurement by the cellular adhesiveness polypeptide (P).

[0041]

The compound (AM) containing the amino group and/or an ammonio radical may be combined with covering material (N). As an approach of combining the compound (AM) containing the amino group and/or an ammonio radical with covering material (N) For example, the compound (AM) containing the amino group and/or an ammonio radical and the approach to which covering material (N) is made to react, The approach of making covering material (N) carrying out physical adsorption of the compound (AM) containing the amino group and/or an ammonio radical to a list etc. is applicable. The approach The chemical association and/or physical adsorption same among the joint approaches of of the cellular adhesiveness polypeptide (P) and covering material (N) which are mentioned later as (1) – (3) can be used, and the same is said also of desirable chemical association and/or desirable physical adsorption, and is said of the quantum approach.

[0042]

The average number (individual) of the amino group of the covering material (N) embellished with the compound (AM) containing the amino group and/or an ammonio radical Two or more [per unit area of the wound cladding material of this invention / 108 //cm] are desirable. It is two or more [1012 //cm] especially preferably, and two or less [1022 //cm] are two or less [1018 //cm] especially preferably two or less [1020 //cm] desirable still more preferably two or more [1010 //cm] still more preferably.

[0043]

Since a cellular adhesiveness polypeptide consists of amino acid as well as protein, it serves as a nutrient of a microorganism and tends to receive mixing of a microorganism. Moreover, in producing a cellular adhesiveness polypeptide by the transgenics microorganism, in order to refine and extract the cellular adhesiveness polypeptide accumulated into the microorganism, the microorganism origin matter is easy to be mixed in a cellular adhesiveness polypeptide. Therefore, it is necessary to manage severely the microorganism to a cellular adhesiveness polypeptide, and mixing of the microorganism origin matter, and to protect them from a wound ingredient (N). As an index of mixing of a microorganism or the microorganism origin matter, endotoxin is suitable at the point which is the harmful matter itself. Endotoxin is a toxin which carries out postmortem isolation from the bacteria which have toxicity in the fungus body constituent itself, and has the basic structure which glycoprotein and a lipid connected (2nd edition Yodosha CO., LTD. issue 2000 of gene engineering keyword book revision).

[0044]

Based on the weight of a cellular adhesiveness polypeptide (P), from a viewpoint of safety, less than 0.15 are desirable still more desirable, and the content (EU/mg) of the endotoxin in the cellular adhesiveness polypeptide (P) of this invention is less than 0.0015 especially preferably less than 0.015.

[0045]

As a measuring method of an endotoxin content, the corpuscle extract of a king crab reacts to endotoxin, and the Limulus test approach using solidifying etc. can be applied. As a reagent kit for Limulus tests which can come to hand easily from a commercial scene, if a trade name shows, RIMURUSU F single Test Wako (Wako Pure Chem industrial company make), RIMURUSU ES-2 single Test Wako (Wako Pure Chem industrial company make), etc. will be mentioned, for example. preparation of the specimen liquid used for a reagent kit — a cellular adhesiveness polypeptide — demineralization distilled water (sterile) and an endotoxinic test — it is carried out by dissolving in the water which endotoxins, such as service water or method water for injection of a station, do not contain. Moreover, as the standard substance, the endotoxin reference standard defined by the Japanese pharmacopoeia and the standard substance authorized with this endotoxin reference standard can be used.

[0046]

As a measuring method of an endotoxin content using a commercial Limulus test reagent kit For example, when the detection sensitivity of endotoxin uses the Limulus test reagent kit of 0.015 EU/mL, 0.2mL(s) of the specimen liquid (1 mg/mL) which dissolved in 1mL of service water are mixed with a LAL reagent. a 1mg test portion (cellular adhesiveness polypeptide) — an endotoxinic test — If the visual judgment of whether the gel of water-insoluble nature is formed is carried out and gel is formed, it can judge with an endotoxin content being 0.015 or more EU/mg, and if gel is not formed, it can judge with an endotoxin content being less than 0.015 EU/mg. Moreover, if it changes to a 1mg test portion and a 0.1mg test portion is used (specimen liquid of 0.1

mg/mL), the judgment of whether to contain 0.15 or more EU/mg or the following similarly can be performed. Moreover, it can judge whether it contains 0.0015 or more EU/mg or the following by using a 10mg test portion similarly (specimen liquid of 10 mg/mL).

[0047]

Since endotoxin is contained in a bacterial cell wall etc., endotoxin may be mixed in a cellular adhesiveness polypeptide, when the cellular adhesiveness polypeptide was manufactured by the gene recombination by bacteria, or when a cellular adhesiveness polypeptide is dealt with except a non-fairy ring boundary. In such a case, the combination of the heating methods to which deactivation of the endotoxin is carried out with heat using a column method, an autoclave, or a hot air sterilizer etc. which separates endotoxin, using an endotoxin adsorption affinity column, a gel filtration column, or the column for hydrophobic chromatographies as an approach of removing the endotoxin mixed in the cellular adhesiveness polypeptide, for example, and these approaches etc. is applicable. Sterilization actuation is simple and the heating method is [among these] sure. [of endotoxin] As heating temperature, 40 degrees C or more 60 degrees C or more are 80 degrees C or more especially preferably desirable still more preferably, and 500 degrees C or less 300 degrees C or less are 200 degrees C or less especially preferably desirable still more preferably. 1 seconds or more are desirable still more desirable, and heating time is 1 minutes or more especially preferably 10 seconds or more, and 5000 or less minutes is 100 or less minutes especially preferably 500 or less minutes desirable still more preferably. The approach of removing such endotoxins can be used for a cellular adhesiveness natural polypeptide (P1) and a cellular adhesiveness artificial polypeptide (P2).

[0048]

The ingredient (following and difficulty biodegradability ingredient (N2)) which can be easily distributed, dissolved or absorbed by neither culture medium nor the wound side can be used for the covering material (N) of this invention at the time of use by the ingredient (henceforth, ready biodegradability ingredient (N1)) which tends to be distributed, dissolved or absorbed by culture medium and the wound side at the time of use by the cell culture, and application to a wound side, and the cell culture, and application to a wound side. Moreover, it can also be used combining a ready biodegradability ingredient (N1) and a difficulty biodegradability ingredient (N2). When applying to a wound side among these ingredients, a stick substitute is easy and a difficulty biodegradability ingredient (N2) is desirable at the point which is easy to deal with it. In addition, the thing containing a toxic substance with the serious bad influence for the body cannot use covering material (N).

[0049]

As a ready biodegradability ingredient (N1), naturally-ocurring polymers (N1A), synthetic macromolecule (N1B), an inorganic substance (N1C), etc. can be used.

As naturally-ocurring polymers (N1A), a collagen, gelatin, glycosaminoglycan, hyaluronic acid, chondroitin sulfate, a keratan sulfate, dermatan sulfate, heparin, an elastin, a chitin, chitosan, a fibrin, an alginic acid, starch, a dextran, albumin, polyhydroxy butanoic acid, pectin, a pectic acid, galactan, a pullulan, agarose, a cellulose, gluten, a fibroin, etc. are mentioned, for example.

[0050]

Lactic-acid, leucine, glycolic-acid, epsilon-caprolactone, and dioxa non, as synthetic macromolecule (N1B), the synthetic polypeptide which does not include the minimum amino acid sequence (X) which expresses a cell adhesion signal to the polymer (polyglycolic acid) which becomes considering the monomer chosen from the group which consists of a malic acid, a lactide, and glycolide as an indispensable monomer (**), and a list is mentioned, for example.

[0051]

As an inorganic substance (N1C), a calcium carbonate, calcium phosphate, etc. are used, for example. Precipitated calcium carbonate, whiting, etc. are mentioned as a calcium carbonate. As calcium phosphate, the mixture of hydroxyapatite, TORIKARUSHIUMU phosphate, and these and other calcium phosphate (for example, mono-calcium hydrogen phosphate etc.) etc. is mentioned.

[0052]

Naturally-ocurring polymers (N1A) and synthetic macromolecule (N1B) are the polymers (polyglycolic acid) which become considering synthetic macromolecule (N1B) and the monomer chosen from the group which consists of lactic-acid, leucine, glycolic-acid, epsilon-caprolactone, and dioxa non, a malic acid, a lactide, and glycolide especially preferably as an indispensable monomer (**) desirable still more preferably among these.

[0053]

As a difficulty biodegradability ingredient (N2), naturally-ocurring polymers (N2A), synthetic macromolecule (N2B), an inorganic substance (N2C), etc. can be used. As naturally-ocurring polymers (N2A), natural fibers (cotton, hair, hemp, silk, etc.) etc. are mentioned, for example.

[0054]

as synthetic macromolecule (N2B) — polyolefine (polyethylene —) olefine copolymers (an ethylene-vinyl acetate copolymer —), such as polypropylene and these denaturation objects An ethylene-ethyl (meta) acrylate

copolymer, an ethylene-methyl (meta) acrylate copolymer, an ethylene-(meta) acrylic-acid copolymer, etc., Polyurethane, polyester, polyacrylic acid, a polyamide, a polyvinyl chloride, A polyvinylidene chloride, polystyrene, a fluororesin, silicone resin, a cellulose and a chemical fiber (viscose rayon and cuprammonium rayon rayon —) polynosic, acetate, triacetate, polyethylene, polypropylene, a polyamide, polyester, the poly acrylic nitril, Vinylon, a polyvinyl chloride, vinylidene, polyurethane, etc. — etc. — it is used. In addition, acrylate (meta) means acrylate and/or methacrylate.

[0055]

As an inorganic substance (N2C), metals (gold, silver, platinum, titanium, nickel, etc.), ceramics (an alumina, a zirconia, alumimium nitride, etc.), etc. are used, for example.

among these — viewpoints, such as handling nature, to a difficulty biodegradability ingredient (N2) — desirable — further — desirable — naturally-ocurring polymers (N2A) and synthetic macromolecule (N2B) — especially — desirable — polyolefine, polyurethane, polyester, and polyacrylic acid — it is polyurethane most preferably.

[0056]

Covering material (N) is chosen synthetically in consideration of flexibility, elasticity, moderate steam permeability, bacillus barrier nature, and sterilization nature. The degree of hardness of covering material (N) is the point that handling is easy, and 40 or more are 60 or more especially preferably 50 or more desirable still more preferably, and 100 or less are 80 or less especially preferably 90 or less desirable still more preferably. In addition, a degree of hardness is measured based on JIS K 6301-1995 and a 5.2 spring-loaded-type hardness test (A form). In order that the moisture vapor transmission (g/m2, 24hr) of covering material (N) may keep the storage in the wound of an exudate moderate, 200 or more are 2000 or more especially preferably 400 or more desirable still more preferably, and 15000 or less are 7000 or less especially preferably 10000 or less desirable still more preferably. In addition, moisture vapor transmission is measured based on JIS Z 0208-1976 (degrees C [40], 90%RH).

[0057]

15 or more are desirable still more desirable than the viewpoint which promotes wound healing, and the contact angle (degree) over the water of the front face of covering material (N) is 50 or more especially preferably 30 or more, and 120 or less are 100 or less especially preferably 110 or less desirable still more preferably. In addition, the contact angle over the water of the front face of covering material (N) can be measured using a contact angle meter (for example, consonance interface science incorporated company make, CA-S150 mold) etc. as a Measuring condition — measurement ambient temperature: — 25**1-degree-C, measurement ambient atmosphere relative humidity:65**5%, and measuring object temperature: — it reads after [of a contact angle] 25**1 degree C, volume:1.8**2microL of a drop, dropping needle:18G of a drop, and reading:dropping 15 **1 second, and comes out. The calculation approach of a contact angle is computed from a degree type.

(Contact angle) =2tan-1{(height of the drop after dropping) / (radius of the drop after dropping)}

[0058]

Although the adsorption treatment by blocking of the physical processing for producing irregularity on the chemical preparation for being able to carry out surface treatment of the contact angle over the water of the front face of covering material (N), and it being able to control covering material (N), for example, giving functional groups (a perfluoroalkyl radical, a polyoxyethylene radical, a carboxyl group, a carbonyl group, a hydroxyl group, amino group, etc.) and a front face, protein, etc. is mentioned, chemical preparation and adsorption treatment are chemical preparation desirable still more preferably among these.

[0059]

As chemical preparation, SG (Soil Guard) processing, SR (Soil Release) processing (a guide to macromolecule drugs, Takehiko Fujimoto editorial supervision, Sanyo Chemical Industries, Ltd. issue), silane coupling agent processing, ozonization, electron ray processing, oxidizer processing, plasma treatment, corona discharge treatment, GORO electrodischarge treatment, etc. can be used, for example. As physical processing, the approach of grinding a front face by the diamond file (DT-101N) etc. can be used, for example, and also it can be made the shape of surface type of a request at the time of molding of covering material (N). The approach of covering material (N) being immersed into a protein content solution, and, for example, making protein adsorbing as adsorption treatment etc. can be used. As protein, milk origin protein, such as blood serum origin protein, such as albumin, and casein, etc. is mentioned.

[0060]

Although there will be especially no limit as a configuration of covering material (N) if it can be used for the therapy of wounds (the bedsore, an ulcer, burn, etc.), for example, the shape of the shape of a sheet and yarn etc. is mentioned, and the shape of the point of the ease of dealing with it in application to a cell culture or a wound to a sheet is [among these] desirable. It is 500 micrometers or less most preferably 3mm or less especially preferably 1cm or less still preferably 5cm or less more preferably [5 micrometers or more 15 micrometers or more are 30 micrometers or more most preferably especially preferably still preferably / 1 micrometers or more / as thickness of a sheet more preferably, and].

[0061]
as a sheet-like gestalt — a film, form (sponge), a nonwoven fabric, and textile fabrics — it knits and cloth, gel, etc. are mentioned. A film and form are films desirable still more preferably among these.
Although the amount of eyes of a film (g/m2) does not have especially a limit, ten or more are 30 or more especially preferably 20 or more desirable still more preferably, and 150 or less are 75 or less especially preferably 100 or less desirable still more preferably. 15 or more are desirable still more desirable, and the consistency (kg/m3) of form is 60 or more especially preferably 40 or more, and 500 or less are 150 or less especially preferably 250 or less desirable still more preferably. Although the amount of eyes of a nonwoven fabric, textiles, and knitting (g/m2) does not have especially a limit, 20 or more are 50 or more especially preferably 30 or more desirable still more preferably, and 300 or less are 100 or less especially preferably 200 or less desirable still more preferably.
[0062]
In addition, in a film and form, you may have the detailed hole at the whole surface or a part. The magnitude of this hole has the desirable magnitude air and whose steam are extent which can be passed easily. As magnitude of this hole, 0.005 or more are 0.05 or more especially preferably 0.01 or more desirable still more preferably as a puncturing area (mm2) of a hole, and 25 or less are five or less especially preferably ten or less desirable still more preferably. Moreover, although a circular, ellipse form, triangle, square, polygon, and line top (slit) etc. is mentioned, as long as the configuration of this hole can keep moderate the storage in the wound of the exudate made into that purpose, it may use which configuration.
[0063]
In the wound cladding material of this invention, a cellular adhesiveness polypeptide (P) and covering material (N) are usually compound-ized by chemical bonds (ionic bond, hydrogen bond, covalent bond, etc.) and/or physical adsorption (adsorption by Van der Waals force). It is the point that a cellular adhesiveness polypeptide (P) and covering material (N) are combined firmly, and a chemical bond is covalent bond desirable still more preferably.
[0064]
As an approach of carrying out covalent bond of the covering material (N) to a cellular adhesiveness polypeptide (P) For example, the thing which has the 1st class amino group or the 2nd class amino group among (1) and (P) for example, an arginine, a histidine, an isoleucine, a leucine, and a methionine — A phenylalanine, threonine, a tryptophan, a thyrosin, a valine, An alanine, an asparagine, an aspartic acid, a glutamine, glutamic acid, A proline, a cysteine, a lysine, a serine, a glycine, an ornithine, a histidine, What has a carboxyl group among the cellular adhesiveness polypeptide which contains 3-aminopropionic acid, 8-amino octanoic acid, 20-amino eicosanoic acid, etc. as a configuration unit, and (N) for example, the synthetic polypeptide which does not include the minimum amino acid sequence (X) showing polyglycolic acid and a cell adhesion signal — Polyethylene or the denaturation object of polypropylene, an ethylene-(meta) acrylic-acid copolymer, Polyacrylic acid, a collagen, gelatin, glycosaminoglycan, hyaluronic acid, Chondroitin sulfate, dermatan sulfate, heparin, an elastin, a fibrin, what has the 1st class amino group or the 2nd class amino group among approach; (2) to which covering material, such as an alginic acid, albumin, pectin, a pectic acid, gluten, and a fibroin, is made to react, and (P), and the thing (for example, polyglycolic acid —) which has hydroxyl among (N) An ethylene-vinyl acetate copolymer, polyurethane, polyester, A cellulose, a chemical fiber, a collagen, gelatin, glycosaminoglycan, Hyaluronic acid, chondroitin sulfate, a keratan sulfate, dermatan sulfate, Heparin, an elastin, a chitin, chitosan, a fibrin, an alginic acid, Starch, a dextran, albumin, polyhydroxy butanoic acid, pectin, What has hydroxyl among approach; (3) to which covering material, such as a pectic acid, galactan, a pullulan, agarose, gluten, and a fibroin, is made to react, and (P) for example, an aspartic acid, glutamic acid, a serine, a threonine, and a thyrosin — What has a halogen atom among approach; (4) to which the cellular adhesiveness polypeptide which contains thyronine, a hydroxy pudding, etc. as a configuration unit, and the thing which has a carboxyl group among (N) are made to react, and (P) What has hydroxyl or a sulfhydryl group among (for example, the cellular adhesiveness polypeptide embellished with the halogenide which has an amino group), and (N) (For example, covering material which has the structure which transposed some or all of hydroxyl to the sulfhydryl group in addition to what has the above-mentioned hydroxyl) approach; made to react — and The approach to which what has a vinyl group among (5) and (P) (for example, cellular adhesiveness polypeptide embellished with the amino-group content monomer), and the thing which has the amino group, hydroxyl, or a carboxyl group among (N) are made to react is mentioned.
[0065]
These reactions can be performed by well-known approaches (for example, approach given in "the foundation of peptide synthesis, an experiment, October 5, Heisei 9, and the Maruzen Co., Ltd. issue" etc.). Specifically, it is as the following (1) - (6).
When making what has the 1st class amino group or the 2nd class amino group among (1) and (P), and the thing which has a carboxyl group among (N) react, After making the carboxyl group of (N) react with a carbodiimide compound beforehand and considering as an acyl iso urea {R'-N=C(OCOR)-NH-R' (part to which -OCOR

originates in (N))}, Amide association can be made to be able to form in (N) and (P) can be made to introduce into it by adding what has the 1st class amino group or the 2nd class amino group among (P). As a carbodiimide compound, N and N'-dicyclohexylcarbodiimide, a 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, etc. are mentioned, for example.

[0066]

When making what has the 1st class amino group or the 2nd class amino group among (2) and (P), and the thing which has hydroxyl among (N) react, The hydroxyl of (N) is made to react with a carbonyldiimidazole compound beforehand. After an imidazoline ring and R make imidazole derivative {R-Im and Im origin} at (N), they can make (P) able to form in (N) and can make N-C association introduce it into it by adding what has the 1st class amino group or the 2nd class amino group among (P). As a carbonyldiimidazole compound, N and N'-carbonyldiimidazole etc. is mentioned, for example.

[0067]

When making what has hydroxyl among (3) and (P), and the thing which has a carboxyl group among (N) react, after making the carboxyl group of (N) react with a carbodiimide compound beforehand and considering as an acyl iso urea, an ester bond can be made to be able to form in (N) and (P) can be made to introduce into it by adding what has hydroxyl among (P).

[0068]

When making what has a halogen atom among (4) and (P), and the thing which has hydroxyl or a sulfhydryl group among (N) react, ether linkage or thioether association can be made to be able to form in (N), and (P) can be made to introduce into it by mixing both under existence of an alkali compound or nonexistence. As an alkali compound, inorganic alkali compounds (a sodium hydroxide, a potassium hydroxide, lithium hydroxide, etc.), organic alkali compounds (a dimethylamino pyridine, ammonia, triethylamine, sodium methoxide, DBU (San Apro trademark), etc.), etc. are mentioned, for example.

[0069]

When making what has a vinyl group among (5) and (P), and the thing which has the amino group, hydroxyl, or a carboxyl group among (N) react, both can be mixed under existence of an alkali compound or nonexistence, and (P) can be made to introduce into (N) by carrying out Michael addition.

[0070]

The approach of supplying a cellular adhesiveness polypeptide (P) to covering material (N), supplying (P) and (N) to a solvent etc., for example as physical adsorption, ionic bond, and/or an approach of carrying out hydrogen bond, mixing, and producing etc. is mentioned. Although there is especially no limit as a solvent, a water solution, water, body fluid, etc. which carry out content of mineral salt, an organic-acid salt, amino acid, a vitamin, alcohol, a lipid and sugar, an acid, and/or the base 0.001 to 50% of the weight (preferably 0.01 - 10 % of the weight) can be used.

[0071]

As mineral salt, a halogenation metal salt, a sulfuric-acid metal salt, a phosphoric-acid metal salt, a nitric-acid metal salt, a carbonic acid metal salt, a fault halogen acid metal, etc. can be used, for example, a sodium chloride, a sodium sulfate, sodium phosphate, a calcium chloride, iron nitrate, potassium chloride, magnesium sulfate, a sodium carbonate, dibasic sodium phosphate, potassium phosphate, a phosphoric-acid hydrogen potassium, a copper sulfate, an iron sulfate, a lithium chloride, a sodium bromide, a lithium bromide, a sodium perchlorate, lithium perchlorate, etc. are mentioned.

As an organic-acid salt, sodium formate, sodium acetate, an acetic-acid lithium, the sodium tartrate, etc. are mentioned, for example.

[0072]

As amino acid, an arginine, a histidine, an isoleucine, a leucine, a methionine, a phenylalanine, threonine, a tryptophan, a thyrosin, a valine, an alanine, an asparagine, an aspartic acid, glutamic acid, a proline, a serine, a glycine, etc. are mentioned, for example.

As a vitamin, a choline, an inositol, nicotinamide, a glutamine, vitamin A, vitamin B12, vitamin C, etc. are mentioned, for example.

As alcohol, the alcohol of carbon numbers 1-4 etc. can be used, for example, a methanol, ethanol, isopropyl alcohol, a butanol, etc. are mentioned.

As a lipid and sugar, a lipid, a monosaccharide, 2 sugar, an oligosaccharide, aminosugar, acid sugar, etc. are mentioned, for example.

[0073]

As an acid, an inorganic acid, the organic acid of carbon numbers 1-6, etc. can be used, for example, a hydrochloric acid, phosphoric acid, an acetic acid, formic acid, a phenol, a sulfuric acid, etc. are mentioned.

As a base, an inorganic base, the organic base of carbon numbers 2-6, etc. can be used, for example, a sodium hydroxide, a potassium hydroxide, ammonia, triethylamine, etc. are mentioned.

As water, distilled water, ion exchange water, tap water, ion-exchange distilled water, etc. are mentioned.

As body fluid, blood, plasma, a blood serum, urine, etc. are mentioned.
It is the water solution with which water contains mineral salt, an acid, and/or a base desirable still more preferably in the water solution containing mineral salt, an acid, and/or a base, and a list in these solvents.
[0074]
The content of the cellular adhesiveness polypeptide in the wound cladding material of this invention (P) Per unit area of a wound cladding material and two or more 0.1 ng/cm are more desirable than the viewpoint which raises cellular adhesiveness. Preferably especially two or more 1 ng/cm still more preferably Two or more 10 ng/cm, It is two or more 100 ng/cm most preferably, and two or less 100 mg/cm is two or less 100microg/cm most preferably two or less 1 mg/cm especially preferably two or less 10 mg/cm still more preferably.
Although especially the measuring method of the content of the cellular adhesiveness polypeptide per unit area (P) is not limited, immunoassay can be used, for example. The content of the cellular adhesiveness polypeptide per unit area (P) can be measured by cutting off a part of front face (for example, 1cmx1cm square configuration) of a wound cladding material, making what carried out the indicator of the enzyme (the following, enzyme labelled antibody 1) specifically react to the antibody combined with a cellular adhesiveness polypeptide (P), and measuring the amount of enzymes of this enzyme labelled antibody 1 that reacted.
An enzyme labelled antibody 1 carries out the chemical bond of an enzyme and the specific antibody, and can usually carry out a chemical bond by the well-known approach. For example, the approach of carrying out the chemical bond of enzymes (for example, a peroxidase, beta-D-galactosidase, alkaline phosphatase, glucose-6-phosphate dehydrogenase, etc.) and the specific antibody by the glutaraldehyde method, the periodic acid method, the maleimide method, the pyridyl disulfide method, etc. is applicable (super-high sensitivity enzyme immunoassay, Eiji Ishikawa work, Japan Scientific Societies Press, Inc., 1993; the volumes enzyme immunoassay, Eiji Ishikawa translation, Tokyo Kagaku Dojin Co., Ltd., and 1989; and enzyme-labeled antibody techniques, and for Keiichi Watanabe, interdisciplinary plan incorporated company, 1992). Moreover, a specific antibody is an antibody specifically combined with a cellular adhesiveness polypeptide (P), and can be produced by the well-known approach. For example, the polyclonal antibody producing method, the monoclonal antibody producing method (the volumes enzyme immunoassay, Eiji Ishikawa translation, Tokyo Kagaku Dojin Co., Ltd., and 1989; and enzyme-labeled antibody techniques, and for Keiichi Watanabe, interdisciplinary plan incorporated company, 1992), etc. are applicable. In addition, the affinity constant to the cross reacting antigen of a specific antibody is so desirable that it is small, for example, when the affinity constant to the cellular adhesiveness polypeptide (P) of a specific antibody is set to 1, one or less is desirable still more desirable, and the affinity constant to a cross reacting antigen is 0.01 or less especially preferably 0.1 or less. These affinity constants can be obtained by the approach of a publication to enzyme immunoassay (an Eiji Ishikawa translation, Tokyo Kagaku Dojin Co., Ltd., 1989).
[0075]
The wound cladding material of this invention may perform sterilization processing if needed. As the sterilization approach, radappertization, ethylene oxide gas sterilization, plasma sterilization, gamma ray sterility, alcoholic sterilization, autoclave sterilization, dry sterilization, etc. are mentioned, for example. Autoclave sterilization and dry sterilization are [among these] desirable at a point with simple sterilization actuation.
As autoclave sterilization and heating temperature in the case of sterilizing by hot air, 40 degrees C or more 60 degrees C or more are 80 degrees C or more especially preferably desirable still more preferably, and 180 degrees C or less 160 degrees C or less are 140 degrees C or less especially preferably desirable still more preferably.
As autoclave sterilization and heating time in the case of sterilizing by hot air, 1 seconds or more are 1 minutes or more especially preferably 10 seconds or more desirable still more preferably, and 5000 or less minutes is 100 or less minutes especially preferably 500 or less minutes desirable still more preferably.
As tub internal pressure in the case of carrying out autoclave sterilization, 0.002 or more MPas are 0.05 or more MPas especially preferably 0.01 or more MPas desirable still more preferably, and 5 or less MPas are 0.2 or less MPas especially preferably 1 or less MPa desirable still more preferably.
[0076]
Since the wound cladding material of this invention promotes wound healing, it is desirable to make a cell growth factor (G1) and/or a cell growth factor cementing material (G2) contain.
The matter which promotes growth of a cell as a cell growth factor (G1) For example, a fibroblast growth factor, a transforming growth factor, an epidermal growth factor, A hepatocyte growth factor, a platelet derived growth factor, insulin like growth factor, a vascular endothelial cell growth factor, A nerve growth factor, a stem cell factor, the leukemia inhibitor, an osteogenesis factor, a heparin joint epidermal growth factor, A neurotrophic factor, a connective tissue growth factor, ANJIOPO ethyne, KONDOROMOJURIN, TENOMOJURIN, interferon, interleukin, a tumor necrosis factor, Bioactive polypeptides, such as a colony stimulating factor, ADORENAMOJURIN, and a natriuresis peptide, etc. are used (it indicates for example, to foundation method name-of-a-person Furuya university publication meeting issue "the tissue engineering edited by the Ueda

fruit" (1999)).
A fibroblast growth factor, a transforming growth factor, an epidermal growth factor, a hepatocyte growth factor, a platelet derived growth factor, insulin like growth factor, a vascular endothelial cell growth factor, an osteogenesis factor, interleukin, and a tumor necrosis factor are a fibroblast growth factor, an epidermal growth factor, insulin like growth factor, a vascular endothelial cell growth factor, interleukin, and a tumor necrosis factor desirable still more preferably from a viewpoint that the range of a tissue cell applicable in these cell growth factors (G1) is wide, and a recovery period can be shortened more.
[0077]
As a cell growth factor cementing material (G2), it is the matter in which a cell growth factor and association by ionic bond etc. are possible, for example, heparin, a heparan sulfate, chondroitin sulfate, dermatan sulfate, a keratan sulfate, hyaluronic acid, gelatin, a collagen, polylactic acid, agarose, an alginic acid, etc. are used (it indicates to for example, foundation method name-of-a-person Furuya university publication meeting issue "the tissue engineering edited by the Ueda fruit" (1999), and Yodosha Issue "the structure of cell adhesion, and a disease" (1998)).
In addition, these alkali-metal salts (a lithium, a potassium, sodium, etc.), alkaline-earth-metal salts (magnesium, calcium, etc.), or ammonium salt is included in heparin, a heparan sulfate, chondroitin sulfate, dermatan sulfate, a keratan sulfate, hyaluronic acid, gelatin, a collagen, polylactic acid, or an alginic acid.
Heparin, a heparan sulfate, chondroitin sulfate, hyaluronic acid, and gelatin are heparin, hyaluronic acid, and gelatin desirable still more preferably from a viewpoint that the range of a tissue cell applicable in these cell growth factor cementing materials is wide, and a recovery period can be shortened more.
[0078]
A cell growth factor (G1) and/or a cell growth factor cementing material (G2) usually exist in the condition of having been combined with covering material (N). The association can use the same chemical bond and/or same physical adsorption as association of the above-mentioned cellular adhesiveness polypeptide (P) and covering material (N), and its same is said also of a desirable chemical bond and/or desirable physical adsorption.
[0079]
The cell growth factor in the wound cladding material of this invention (G1), and/or the content of a cell growth factor cementing material (G2) Per unit area of the wound cladding material of the viewpoint of compaction of a recovery period to this invention, Two or more 0.01 pg/cm desirable still more preferably Two or more 0.1 pg/cm, It is two or more 10 pg/cm most preferably, and two or less 100microg/cm is two or less 0.1microg/cm most preferably two or less 1microg/cm especially preferably two or less 10microg/cm still more preferably two or more 1 pg/cm especially preferably. In addition, it is one or less most preferably ten or less especially preferably 100 or less still preferably [1000 or less] more preferably [are 0.01 or more most preferably 0.001 or more especially preferably 0.0001 or more still more preferably / when it contains a cell growth factor (G1) and cell growth factor cementing material (G2) / these content ratio (G1/G2) is / 0.00001 or more / desirable, and /, and].
Although especially the measuring method of the cell growth factor per unit area (G1) and/or the content of a cell growth factor cementing material (G2) is not limited, immunoassay can be used, for example. By making what carried out the indicator of the enzyme (the following, enzyme labelled antibody 2) react to the antibody which cuts off a part of front face (the 1cmx1cm shape of for example, a square) of a wound cladding material with and/or (G1) (G2), and is specifically combined, and measuring the amount of enzymes of this enzyme labelled antibody 2 that reacted, it is per unit area (G1), and and/or (G2) can measure a content. In addition, an enzyme labelled antibody 2 is producible like the above-mentioned enzyme labelled antibody 1.
[Example]
[0080]
Although an example is hung up over below and this invention is explained to it in more detail, this invention is not limited only to these examples.
<Example 1>
Preparation of a cellular adhesiveness polypeptide [P2-1]
According to the approach of given [in a ****** No. 502935 / three to / official report] in an example, it has respectively an Arg Gly Asp array and nine (Gly Ala Gly Ala Gly Ser) arrays [about 13] (40), peptide "SLPF" of number average molecular weight 100,000 [about] was manufactured with transgenics Escherichia coli, the column chromatography refined, and the cellular adhesiveness polypeptide [P2-0] was obtained. Furthermore, the cellular adhesiveness polypeptide [P2-1] was obtained by carrying out autoclave sterilization (120 degrees C, 20 minutes) of this [P2-0].
[0081]
Preparation of a difficulty biodegradability ingredient [N2]
6.67g of aquosity urethane (trade name: made in [Sanyo Chemical Industries, Ltd.] Parma Lynn UA200) and 3.33g of ion exchange water were mixed, and the urethane water solution was prepared. This urethane water

solution was thrown in on the polypropylene sheet (incorporated company medical agent make) with a 20cm [20cm by] x height of 1mm, and was left in the room temperature (about 25 degrees C). 24 hours after room temperature neglect, 120 degrees C dried in the fair wind dryer for 1 hour. The urethane film formed on the polypropylene sheet was exfoliated from the polypropylene sheet after desiccation, and the difficulty biodegradability ingredient [N2] was obtained.

[0082]

Preparation of a wound cladding material [S1]

1mg of a cellular adhesiveness polypeptide [P2-1] was dissolved in 1mL of a 4.5M lithium perchlorate water solution, it diluted with the phosphate buffer solution (following, PBS) of 0.02M and pH7.2 which contains 99.5% of sodium chloride at 0.85 % of the weight 20 times further, and P2-1 water solution (50microg/mL) was produced. 10cmx10cm of the 50mL(s) and the difficulty biodegradability ingredient [N2] of this P2-1 water solution was supplied to the glass petri dish, standing was carried out at 25 degrees C for 1 hour, and [P2-1] was made to stick to [N2]. Finally the ion exchange water of 100mL(s) washed [N2] to which [P2-1] stuck 5 times, it was made to dry in a 37-degree C fair wind dryer for 12 hours, and the wound cladding material [S1] was prepared. (Coating weight of P2-1 (content): 0.5microg/cm2)

[0083]

The coating weight (content) of the cellular adhesiveness polypeptide [P2-1] of a wound cladding material [S1] was measured in the following procedures.

(1) The known standard wound cladding material [H1] and the wound cladding material [S1] with strange coating weight were respectively cut off in the 1cmx1cm square configuration, the coating weight (content) of a cellular adhesiveness polypeptide [P2-1] supplied one sheet in 3mL(s) of PBS which contains cow serum albumin at 1 % of the weight, and it was immersed at the room temperature (25 degrees C) for 2 hours.

In addition, although preparation of a standard wound cladding material was performed like preparation of the above-mentioned wound cladding material [S1], coating weight condensed P2-1 water solution after a cellular adhesiveness polypeptide adhering, freeze-dried, found non-adhered cellular adhesiveness polypeptide weight, and found it by deducting non-adhered cellular adhesiveness polypeptide weight from the cellular adhesiveness polypeptide weight before adhesion.

[0084]

(2) Each wound cladding material was taken out, each one wound cladding material was thrown in in 2mL(s) of PBS which contains peroxidase-labeling anti-P2-1 antibody by 10microg/mL, and contains 1 % of the weight and Tween20 for cow serum albumin at 0.2 % of the weight, and it reacted at 37 degrees C for 2 hours. Each wound cladding material was washed 3 times after the reaction by 5mL(s) of PBS which contains Tween20 at 0.2 % of the weight.

In addition, preparation of peroxidase-labeling anti-P2-1 antibody the polyclonal antibody producing method (enzyme immunoassay and an Eiji Ishikawa translation --) According to Tokyo Kagaku Dojin Co., Ltd. and 1989, **** a cellular adhesiveness polypeptide [P2-1] to a rabbit, and anti-P2-1 antibody is obtained. By combining the anti-P2-1 antibody and peroxidase (Toyobo Co., Ltd. make) by the maleimide method (enzyme immunoassay, an Eiji Ishikawa translation, Tokyo Kagaku Dojin Co., Ltd., 1989), peroxidase-labeling anti-P2-1 antibody was obtained.

[0085]

(3) Each wound cladding material was taken out, each one wound cladding material was thrown in in the mixed liquor of 0.2mL(s) of the coloring liquid set (Sanyo Chemical Industries, Ltd. make) of the reagent only for OLYDAS(s), and 1.8mL(s) of ion exchange water, and it reacted at 37 degrees C for 1 hour. Spectrometry was carried out on the wavelength of 380nm after the reaction.

(4) The calibration curve was created using the absorbance of a standard wound cladding material [H1], and the coating weight of a wound cladding material [S1] was obtained from the calibration curve. Hereafter, the coating weight of a polypeptide was measured similarly.

[0086]

<Example 2>

Preparation of a wound cladding material [S2]

0.479g of a 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (sigma company make) was dissolved in the ion exchange water of 50mL(s), and the carbodiimide water solution was produced. 10cmx10cm of the 50mL(s) and the difficulty biodegradability ingredient [N2] of this carbodiimide water solution was supplied to the glass petri dish, and it put at 25 degrees C for 1 hour. Then, the ion exchange water of 100mL washed 5 times. Next, 50mL(s) of P2-1 water solution were supplied, standing was carried out at 25 degrees C for 1 hour, and [P2-1] was combined with [N2]. Finally the ion exchange water of 100mL(s) washed [N2] which [P2-1] combined 5 times, it was made to dry in a 37-degree C fair wind dryer for 12 hours, and the wound cladding material [S2] was prepared. (Coating weight of P2-1 (content): 1microg/cm2)

[0087]

<Example 3>
Preparation of a wound cladding material [S3]
0.479g of a 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (sigma company make) was dissolved in the ion exchange water of 50mL(s), and the carbodiimide water solution was produced. 10cmx10cm of the 50mL(s) and the difficulty biodegradability ingredient [N2] of this carbodiimide water solution was supplied to the glass petri dish, and it put at 25 degrees C for 1 hour. Then, the ion exchange water of 100mL washed 5 times. Next, 50mL(s) of P2-1 water solution were supplied, standing was carried out at 25 degrees C for 1 hour, and [P2-1] was combined with [N2]. Then, the ion exchange water of 100mL washed 5 times. Furthermore, cell growth factor content [water-solution GS 11] 50mL which contains the fibroblast growth factor (product made from BEKUTONDIKKINSON) [G1-1] which is a cell growth factor (G1) by 50 ng/mL was supplied, standing was carried out at 25 degrees C for 1 hour, and [G1-1] was combined with [N2]. Then, the ion exchange water of 100mL washed 5 times, and the wound cladding material [S3] was prepared. (Coating weight of P2-1: 1microg/cm2)
[0088]
<Example 4>
Preparation of a wound cladding material [S4]
The cell growth factor content water solution [GS12] which contains the interleukin 2 (product made from BEKUTONDIKKINSON) [G1-2] which is a cell growth factor (G1) by 5 ng/mL instead of the cell growth factor content water solution [GS11] of an example 3 was used, and the wound cladding material [S4] was prepared like the example 3. (Coating weight of P2-1: 1microg/cm2)
[0089]
<Example 5>
Preparation of a wound cladding material [S5]
The cell growth factor cementing material content water solution [GS21] which contains the heparin sodium (Nakarai Tesuku, Inc. make) [G2-1] which is a cell growth factor cementing material (G2) by 50 ng/mL instead of the cell growth factor content water solution [GS11] of an example 3 was used, and the wound cladding material [S5] was prepared like the example 3. (Coating weight of P2-1: 1microg/cm2)
[0090]
<Example 6>
Preparation of a wound cladding material [S6]
The cell growth factor cementing material content water solution [GS22] which contains the hyaluronic acid [G2-2] (ICN biotechnology medical company make) which is a cell growth factor cementing material by 50 ng/mL instead of the cell growth factor content water solution [GS11] of an example 3 was used, and the wound cladding material [S6] was prepared like the example 3. (Coating weight of P2-1: 1microg/cm2)
[0091]
<Example 7>
Preparation of a cellular adhesiveness polypeptide [P2-2]
Cellular adhesiveness polypeptide [P2-1]10mg obtained in the example 1 was supplied to what dissolved 0.096g of N and N'-carbonyldiimidazole (Wako Pure Chem, Inc. make) in dimethyl sulfoxide 5mL, and it was made to react to it for 10 minutes at 37 degrees C.
Next, ethylenediamine 0.107g was added and it was made to react at 37 degrees C for 20 hours. It supplied to the dialysis tube after the reaction, the ion exchange water of 1L performed dialysis of 2 hours 5 times, and the cellular adhesiveness polypeptide [P2-2] was obtained. (Number:60 piece /, molecule of the amino group with which P2-2 were embellished)
[0092]
In addition, the number of the amino group with which P2-2 were embellished was measured in the following procedures.
(1) 0mg of L-lysine, 10mg, 30mg, and 100mg were respectively dissolved in 1L of the carbonic acid buffer solution (following, CB) of 0.6M and pH9.5, and it considered as the standard series. Moreover, 1mg of P2-1 and P2-2 was respectively dissolved in 1mL of CB, and it considered as specimen liquid 1 and specimen liquid 2, respectively.
(2) A standard series, specimen liquid 1, and specimen liquid 2 were respectively thrown in with 100microL / test tube in the glass test tube, the water solution which contains 2,4,6-trinitrobenzenesulfonic acid sodium (TNBS) at 7.2mg % of the weight further was added and stirred with 20microL / test tube, and it was left at the room temperature (25 degrees C) for 2 hours.
(3) Ion exchange water was added with 1mL / test tube 2 hours after, and spectrometry was carried out by 367nm.
(4) The absorbance of a standard series, the number of the amino group, and a calibration curve were created, and from the calibration curve, the number of the amino group of specimen liquid 1 and specimen liquid 2 was

computed, and it considered as the number of the amino group embellished with deducting the number of specimen liquid 1 from the number of specimen liquid 2. The quantum of the number of the amino group was carried out like the following.
[0093]
Preparation of a wound cladding material [S7]
Instead of P2-1 water solution of an example 2, PBS (P2-2 water solution) which contains a cellular adhesiveness polypeptide [P2-2] by 50microg/mL was used, and the wound cladding material [S7] was prepared like the example 2. (Coating weight of P2-2: 1microg/cm2)
[0094]
<Example 8>
Preparation of a wound cladding material [S11]
Instead of the cell growth factor content water solution [GS11] of an example 3, the polyethyleneimine water solution [GS13] which contains polyethyleneimine (the Wako Pure Chem Industries make) by 100microg/mL was used, and the wound cladding material [S11] was prepared like the example 3. (Coating weight of P2-1: 1microg/cm2)
[0095]
<Example 9>
Preparation of a cellular adhesiveness polypeptide [P2-3]
Cellular adhesiveness polypeptide [P2-1]10mg obtained in the example 1 was supplied to what dissolved 0.096g of N and N'-carbonyldiimidazole (Wako Pure Chem, Inc. make) in dimethyl sulfoxide 5mL, and it was made to react to it for 10 minutes at 37 degrees C. Next, polyethyleneimine 0.357g was added and it was made to react at 37 degrees C for 20 hours. It supplied to the dialysis tube after the reaction, the ion exchange water of 1L performed dialysis of 2 hours 5 times, and the cellular adhesiveness polypeptide [P2-3] was obtained. (Number:three piece /, molecule of the amino group with which P2-3 were embellished)
[0096]
Preparation of a wound cladding material [S12]
Instead of P2-1 water solution of an example 2, PBS (P2-3 water solution) which contains a cellular adhesiveness polypeptide [P2-3] by 100microg/mL was used, and the wound cladding material [S12] was prepared like the example 2. (Coating weight of P2-3: 1microg/cm2)
[0097]
<The example 1 of a comparison>
Preparation of a wound cladding material [S8]
The difficulty biodegradability ingredient [N2] of an example 1 was used as the wound cladding material [S8] as it was.
[0098]
<The example 2 of a comparison>
Preparation of a wound cladding material [S9]
The collagen film (about 1mm in the Koken Co., Ltd. make, thickness) was used as the wound cladding material [S9] as it was.
[0099]
<The example 3 of a comparison>
Preparation of a wound cladding material [S10]
In "preparation of a wound cladding material [S1]" of an example 1, except omitting the autoclave sterilization of a cellular adhesiveness polypeptide [P2-0], it prepared like the example 1 and the wound cladding material [S10] was prepared.
[0100]
<Evaluation 1 (endotoxin concentration)>
The cellular adhesiveness polypeptide [P2-0], the cellular adhesiveness polypeptide [P2-1], the cellular adhesiveness polypeptide [P2-2], and the cellular adhesiveness polypeptide [P2-3] were made into the test portion for endotoxin content measurement. About each of this test portion, 1mg and 0.1mg were supplied to demineralization distilled water (sterile) (Wako Pure Chem Industries make) 1mL, and the specimen liquid of 1 mg/mL and 0.1 mg/mL was prepared. Moreover, the detection sensitivity of endotoxin measured specimen liquid according to the directions for use of a measurement kit, using RIMURUSU ES-2 single Test Wako (Wako Pure Chem industrial company make) of 0.015 EU/mL as a measurement kit.
[0101]
<Evaluation result 1>
Gel was formed for both specimen liquid of 1 mg/mL and 0.1 mg/mL (endotoxin positivity), and the endotoxin contents of a test portion of the cellular adhesiveness polypeptide [P2-0] were 0.015EU / 0.1mg or more, i.e., 0.15 EU/mg. [or more]

Gel was not formed for both specimen liquid of 1 mg/mL and 0.1 mg/mL (endotoxin negative), but the endotoxin content of a test portion of a cellular adhesiveness polypeptide [P2-1], [P2-2], and [P2-3] was less than 0.015 EU/mg.

[0102]

<Evaluation 2 (cell culture A)>

What cut off respectively wound cladding material [of examples 1-7 and the examples 1-3 of a comparison] [S1] - [S10] in 1cmx1cm magnitude was supplied to the base of 24 hole polystyrene plate (product made from BEKUTONDIKKINSON) in one sheet / hole, and after sticking the four corners of a wound cladding material on a base on a vinyl tape, in the clean bench, UV irradiation was performed for 8 hours and it sterilized. In addition, about one kind of wound cladding material, four holes were used and the same actuation was performed by four holes. It is the same as that of the following.

next, the object for normal Homo sapiens skin fibroblast growth -- low -- blood serum culture-medium (Kurabo Industries, Ltd. make) 1mL and 20,000 normal Homo sapiens skin fibroblasts (Kurabo Industries, Ltd. make) were supplied to 24 hole polystyrene plate 1 hole, and the cell culture for three days was performed in the incubator of 37 degrees C and CO2 concentration 5 capacity %.

Three days after culture, wound cladding material [S1] - [S10] is respectively removed from the base of a plate. Wound cladding material [S1] - [S10] is supplied to the base of new 24 hole polystyrene plate in one sheet / hole. The trypsin solution (the solution by which the 0.25g trypsin is dissolved into 100mL, trade name:Trypsin-EDTA, product made from in vitro JIEN, Inc.) of 0.25 weight / capacity % was fed into the pan in 200microL / hole, and it was left for 3 minutes at 25 degrees C.

It was made mixed liquor by supplying fetal calf serum (product made from Gibco BRL) in 20microL / hole, and carrying out pumping with a pipet after 3 minutes. 20microL of the mixed liquor, 30microL of the phosphate buffer solution (0.02M, pH7.2) which contains NaCl at 0.85 % of the weight, and 10microL of tetra-color one (Seikagaku make) were supplied to one hole of 96 hole polystyrene plate (product made from BEKUTONDIKKINSON), and were left in the incubator of 37 degrees C and CO2 concentration 5 capacity % for 4 hours.

4 hours after, the spectrophotometer was used, the amount of formazan generation was measured with the absorbance of 492nm (contrast wavelength of 630nm), and this value was made into cell activity. Cell activity is proportional to the height of the absorbance concerned. These results are shown in Table 1 (these results are average data for four holes respectively.). In addition, the tetrazolium salt of tetra-color one is returned by the dehydrogenase of an intracellular mitochondrion, and it colors by generating formazan. Moreover, removing a wound cladding material of the wound cladding material [S9] of the example 2 of a comparison from the base of a plate three days after culture was not completed by collapse of a configuration, and it was not able to measure cell activity.

[0103]

[Table 1]

	創傷被覆材	細胞活性 (λ=492nmの吸光度)
実施例1	創傷被覆材［S1］	0．21
実施例2	創傷被覆材［S2］	0．3
実施例3	創傷被覆材［S3］	0．44
実施例4	創傷被覆材［S4］	0．33
実施例5	創傷被覆材［S5］	0．37
実施例6	創傷被覆材［S6］	0．37
実施例7	創傷被覆材［S7］	0．4
比較例1	創傷被覆材［S8］	0．14
比較例2	創傷被覆材［S9］	測定不能
比較例3	創傷被覆材［S10］	0．18

[0104]

From the result of Table 1, the wound cladding materials S1-S7 of this invention are understood that the cell

activity of the cell pasted up on a wound cladding material is very high compared with the wound cladding materials S8-S10 of the example of a comparison. Moreover, collapse of a configuration [like the example 2 of a comparison] whose wound cladding material of this invention is was not produced.

[0105]

<Evaluation 3 (cell culture B)>

Each of the wound cladding material [S2] of examples 2, 8, and 9 and the example 1 of a comparison, [S11], [S12], and [S8] was cut off in the magnitude of 2 the diameter of 1cm, and the line sterilized UV irradiation in the clean bench for 8 hours.

On the other hand, according to the operation manual of this kit, culture dermis was produced using PreTissue-Dermal (Toyobo Co., Ltd. make) of a three-dimensions culture organization construction kit. Culture-medium exchange of the blood serum culture medium currently used by this kit was carried out after production to the DMEM culture medium (product made from ICN Biomedicals) which is a serum free medium, and it considered as the culture dermis under a non-blood serum environment.

The above-mentioned wound cladding material [S2], [S11], [S12], and [S8] were put on the top face of this culture dermis, and the cell culture for five days was performed in the incubator of 37 degrees C and CO2 concentration 5 capacity %. Five days after culture, a wound cladding material [S2], [S11], [S12], and [S8] are respectively removed from the top face of culture dermis. On the base of new 24 hole polystyrene plate, a wound cladding material [S2], [S11], [S12] and [S8] were supplied in one sheet / hole, further, 125microL / hole, and tetra-color one (Seikagaku make) were supplied in 25microL / hole, and PBS was left in the incubator of 37 degrees C and CO2 concentration 5 capacity % for 4 hours. 4 hours after, the spectrophotometer was used, the amount of formazan generation was measured with the absorbance of 492nm (contrast wavelength of 630nm), and this value was made into the cell activity 2. The cell activity 2 is proportional to the height of the absorbance concerned. These results are shown in Table 2 (these results are average data for four holes respectively.).

[0106]

[Table 2]

	創傷被覆材	細胞活性２ （λ=492nmの吸光度）
実施例２	創傷被覆材［Ｓ２］	０．０５０
実施例８	創傷被覆材［Ｓ１１］	０．１１７
実施例９	創傷被覆材［Ｓ１２］	０．０７１
比較例１	創傷被覆材［Ｓ８］	０．０３９

[0107]

From the result of Table 2, the wound cladding materials S2, S11, and S12 of this invention are understood that the cell activity of the cell by which migration is carried out to a wound cladding material from culture dermis is very high compared with the wound cladding material S8 of the example of a comparison.

[0108]

<Evaluation 4 (animal experiment A)>

To DM mouse (C57 BLK Jcl db/db, made in Japanese Clare, Inc.), inhalation-of-air anesthesia by diethylether was carried out, the whole regions-of-back surface was shaved using the feather razor, and all layer skin loss wounds [that it is circular in the center section (diameter of 1cm)] were produced. In addition, that by which the onset of diabetes mellitus is checked was used for DM mouse using the strip for a glycosuria check (UROPISU, Fujisawa Pharmaceutical Co., Ltd. make).

What cut off respectively the wound cladding material [S2] of an example 2, the wound cladding material [S8] of the example 1 of a comparison, and the wound cladding material [S9] of the example 2 of a comparison in 2cmx2cm magnitude On an adhesion film (trade name: multi-fix, product made from ARUKEA, Inc.), lamination, It stuck and carried out so that a wound cladding material side might hit this wound surface, the absorbent cotton for raising adhesion with a wound surface further was piled up, and by the adhesive bandage (trade name: silky tex, product made from ARUKEA, Inc.), the truncus section perimeter was twisted and it fixed.

The growth environment made the room temperature of 24 degrees C, feed, and water supply the free intake condition. As the 14th day, the viewpoint removed the wound therapy ingredient from the wound surface on the 14th, extracted the whole wound including the target neoformation from the laboratory animal including the tunica muscularis which exists under neoformation, carried out immobilization and paraffin embedding processing, and produced the organization intercept. Hematoxylin and eosin stain (HE staining) processing of the produced

organization intercept was carried out, and neoformation was evaluated. The photograph of this neoformation is shown in drawing 1 (wound cladding material [S2]) and drawing 2 (wound cladding material [S8]).
In addition, in the macro-scopic view, although infection had not arisen in the wound surface using a wound cladding material [S2] and a wound cladding material [S8], infection had arisen in the wound surface using a wound cladding material [S9]. Moreover, organization extraction was impossible for the wound cladding material [S9] by conglutination with an organization.
From drawing 1 , much neoformation and many cellular infiltration are seen and the thing using the wound cladding material [S2] of this invention of an example 2 can be said to be the good playback condition of accepting matrix production of a playback in-house so much.
From drawing 2 , the thing using the wound cladding material [S8] of the example 1 of a comparison is deficient in neoformation and the cellular infiltration, and a playback condition can be said to be a defect.
[0109]
<Evaluation 5 (animal experiment B)>
(1) Application of the wound cladding material [S11] of an example 8
Replace with a wound cladding material [S2], [S8], and [S9], and the wound cladding material [S11] prepared in the example 8 is used. Except having changed having changed the magnitude of all layer skin loss wounds into the diameter of 1.4cm from the diameter of 1cm, and an adhesion film into BAIOKURUSHIBU (trade name) by Johnson & Johnson Like evaluation 4 (animal experiment A), DM mouse was grown and the animal experiment of wound healing was started. In addition, the wound cladding material was removed from the wound surface after initiation on the 3rd, and the wound cladding material [S11] was again applied with the adhesion film.
(2) Application of the wound cladding material [S8] of the example 1 of a comparison, and a trafermin solution : 250microg of the Fiblast spray 250 (Kaken Pharmaceutical Co., Ltd. make) which are the bedsore and skin ulcer therapy agent of drugs was dissolved in the physiological saline of 2.5mL(s), and the trafermin solution of 100microg/mL was prepared.
It replaced with the wound cladding material [S11], and before making a wound cladding material rival with an adhesion film using the wound cladding material [S8] prepared in the example 1 of a comparison, except 0.2mL(s) (equivalent to trafermin 20microg) of a trafermin solution being dropped at all the above-mentioned layer skin loss wounds with a pipet, like the above (1), DM mouse was raised and the animal experiment of wound healing was started.
In addition, after removing a wound cladding material from a wound surface after initiation on the 3rd, trafermin solution 0.2mL was dropped at the loss wound, and the wound cladding material [S8] was again applied with the adhesion film.
(3) Observation of a wound healing condition
As the 7th day and the 14th day, the observation day removed the wound cladding material from the wound surface on the observation day, and performed macro-scopic observation of a wound surface. The photograph is shown in drawing 3 (the 7th day, wound cladding material [S11]), drawing 4 (the 7th day, a wound cladding material [S8], and trafermin solution), drawing 5 (the 14th day, wound cladding material [S11]), and drawing 6 (the 14th day, a wound cladding material [S8], and trafermin solution).
As for the thing using the wound cladding material [S11] of this invention of an example 8 as a result of the 7th day, the epidermination from a lip-of-wound perimeter enclosure was accepted (drawing 3). On the other hand, as for the thing using the wound cladding material [S8] and trafermin solution of the example 1 of a comparison, epidermination was not accepted (drawing 4).
As for the thing using the wound cladding material [S11] of this invention of an example 8 as a result of the 14th day, epidermination was accepted in the whole wound surface (drawing 5). On the other hand, although epidermination was accepted in a part of wound surface, as for the thing using the wound cladding material [S8] and trafermin solution of the example 1 of a comparison, epidermination was not accepted in the whole wound surface (drawing 6).
[Availability on industry]
[0110]
The wound cladding material of this invention demonstrates a good wound curative effect, when the cell contained in the exudate which exudes from the cell contained in ****** etc. or a wound pastes the wound cladding material of this invention. Effectiveness especially wonderful in respect of epidermination is shown.
As a cell which can be pasted up on the wound cladding material of this invention the cell (an epithelial cell —) which the cell of the Homo sapiens origin is suitable, for example, participates in the skin cells (a vascular endothelial cell —) which participate in a blood vessel, such as fibroblast, a vascular endothelial cell, and a smooth muscle cell Cells which participate in muscles, such as a smooth muscle cell and fibroblast (muscular cell etc.), The cells (fat cell etc.) which participate in a fat, the cell which participates in a nerve (nerve cell etc.), The cells (hepatocyte etc.) which participate in liver, the cell which participates in the pancreas (pancreas RA islet cell etc.), The cell which participates in the kidney (a kidney epithelial cell, a proximal tubule epithelial cell,

mesangial cell, etc.), The cell which participates in lungs and a bronchial tube (an epithelial cell, fibroblast, a vascular endothelial cell, smooth muscle cell, etc.), the cells (a visual cell, a cornea epithelial cell, cornea endothelial cell, etc.) which participate in an eye, and the cell (an epithelial cell —) which participates in a prostate gland Cells which participate in a bone, such as an interstitial cell and a smooth muscle cell (osteoblast, osteocyte, osteoclast, etc.), the cells (a chondroblast, chondrocyte, etc.) which participate in a cartilage, the cells (a periodontium cell, osteoblast, etc.) which participate in a gear tooth, the cells (a leucocyte, erythrocyte, etc.) which participate in blood, and a stem cell — {— for example A bone marrow undifferentiated mesenchyme system stem cell, a skeletal muscle stem cell, a hematopoietic-system stem cell, a neural stem cell, A liver stem cell (oval cell, small hepatocyte, etc.), A fat tissue stem cell, an embryonal trunk (ES) cell, an epidermis stem cell, an intestinal tract stem cell, a sperm stem cell, }, such as a germ reproduction trunk (EG) cell, pancreas stem cells (pancreatic duct epithelium stem cell etc.), a leucocyte system stem cell, a lymphoid stem cell, a cornea system stem cell, and precursor cells (a fat precursor cell, a blood vessel inner-bark precursor cell, a cartilage precursor cell, a lymphocytic-series precursor cell, NK precursor cell, etc.), etc. is mentioned.

[0111]

As application to the wound side of the wound cladding material of this invention, if it can cover to a wound side, it can apply without a limit, for example, you may fix using covering, dressings, an elastic mesh, or an eyepatch with adhesives or a binder etc., and may suture or paste up. Moreover, in the case of chronic wounds, such as an ulcer, application to the wound of a yellow term to a red term has a desirable wound, and, in the case of acute wounds, such as a burn and a traumatic skin deficiency, the application to the wound of a granulation formative period from an inflammation term has a desirable wound.

Furthermore, the wound cladding material of this invention is applicable also as a base material for cultivating the above-mentioned cell outside a body.

[Brief Description of the Drawings]

[0112]

[Drawing 1] It is the microphotography (one 45 times the scale factor of this) of the organization intercept in the <evaluation 4 (animal experiment A)> using the wound cladding material [S2] of this invention obtained in the example 2.

[Drawing 2] It is the microphotography (one 45 times the scale factor of this) of the organization intercept in the <evaluation 4 (animal experiment A)> using the wound cladding material for a comparison [S8] obtained in the example 1 of a comparison.

[Drawing 3] It is the photograph (one 3 times the scale factor of this) of the wound surface on the 7th in the <evaluation 5 (animal experiment B)> using the wound cladding material [S11] of this invention obtained in the example 8.

[Drawing 4] It is the photograph (one 3 times the scale factor of this) of the wound surface on the 7th in the <evaluation 5 (animal experiment B)> using the wound cladding material for a comparison [S8] obtained in the example 1 of a comparison.

[Drawing 5] It is the photograph (one 3 times the scale factor of this) of the wound surface on the 14th in the <evaluation 5 (animal experiment B)> using the wound cladding material [S11] of this invention obtained in the example 8.

[Drawing 6] It is the photograph (one 3 times the scale factor of this) of the wound surface on the 14th in the <evaluation 5 (animal experiment B)> using the wound cladding material for a comparison [S8] obtained in the example 1 of a comparison.

[Translation done.]

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(54)【発明の名称】　ポリペプチド含有基材

(57)【要約】
【課題】　細胞の接着性及び増殖性が高く、特に無血清培地でも効率的に培養できる基材を提供すること。
【解決手段】　細胞接着シグナルを現す最小アミノ酸配列を１分子中に少なくとも１個有するポリペプチド（Ｐ）を含んでなり、かつ２級アミノ基（Ａ１）、３級アミノ基（Ａ２）、アンモニオ基（Ａ３）、ホスファチジル基（Ａ４）及びリゾホスファチジル基（Ａ５）からなる群より選ばれる少なくとも１つの官能基（Ａ）、並びに／又は糖（Ｂ１）及びステロイド環（Ｂ２）から選ばれる少なくとも１つの構造（Ｂ）を有してなることを特徴とするポリペプチド含有基材を用いる。

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【特許請求の範囲】

【請求項１】 細胞接着シグナルを現す最小アミノ酸配列を１分子中に少なくとも１個有するポリペプチド（Ｐ）を含んでなり、かつ２級アミノ基（Ａ１）、３級アミノ基（Ａ２）、アンモニオ基（Ａ３）、ホスファチジル基（Ａ４）及びリゾホスファチジル基（Ａ５）からなる群より選ばれる少なくとも１つの官能基（Ａ）、並びに／又は糖（Ｂ１）及びステロイド環（Ｂ２）から選ばれる少なくとも１つの構造（Ｂ）を有してなることを特徴とするポリペプチド含有基材。

【請求項２】 ポリペプチド（Ｐ）と官能基（Ａ）及び／又は構造（Ｂ）との全て又は一部が基材表面に存在してなる請求項１記載のポリペプチド含有基材。

【請求項３】 ポリペプチド（Ｐ）の含有量（μｇ／ｃｍ²）が基材の表面積１ｃｍ²当り０．１以上１００以下である請求項１又は２に記載のポリペプチド含有基材。

【請求項４】 ポリペプチド（Ｐ）の細胞接着シグナルを現す最小アミノ酸配列の数が、（Ｐ）１分子中に３～５０個である請求項１～３のいずれかに記載のポリペプチド含有基材。

【請求項５】 ポリペプチド（Ｐ）の細胞接着シグナルを現す最小アミノ酸配列が、Arg Gly Asp配列、Leu Asp Val配列、Arg Glu Asp Val配列（１）、TyrIle Gly Ser Arg配列（２）、Pro Asp Ser Gly Arg配列（３）、Arg Tyr Val Val Leu Pro Arg配列（４）、Leu Gly Thr Ile Pro Gly配列（５）、Arg Asn IleAla Glu Ile Ile Lys Asp Ile配列（６）、Ile Lys Val Ala Val配列（７）、Leu Arg Glu配列、Asp Gly Glu Ala配列（８）及びHis Ala Val配列からなる群より選ばれる少なくとも１種の配列である請求項１～４のいずれかに記載のポリペプチド含有基材。

【請求項６】 官能基（Ａ）及び／又は構造（Ｂ）の含有量が基材の表面積１ｃｍ²当り１×１０¹³～1×１０²⁰個である請求項１～５のいずれかに記載のポリペプチド含有基材。

【請求項７】 官能基（Ａ）が、ジメチルアミノ基、トリメチルアンモニオ基、ジエチルアミノ基、ジエチルメチルアンモニオ基及び／若しくはグアニジノ基、並びに／又はジシアンジアミドとホルマリンとの重縮合物、ジシアンジアミドとジエチレントリアミンとの重縮合物、エピクロルヒドリンとジメチルアミンとの付加重合物、ジメチルジアリルアミンモニウムクロリド重合物及びジアリルアミン塩酸塩と二酸化硫黄との共重合物からなる群より選ばれる少なくとも１つの重合体に含まれる２級アミノ基、３級アミノ基、アンモニオ基、イミノ基、アミジノ基及び／若しくはグアジノ基である請求項１～６のいずれかに記載のポリペプチド含有基材。

【請求項８】 基材が、体積平均粒子径（μｍ）が５０以上１００以下、かつ密度（ｇ／ｃｍ³）が１．０２以上１．０４以下の微粒子である請求項１～７のいずれかに記載のポリペプチド含有基材。

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【請求項９】 スチレン及び多官能性モノマーを必須構成単量体としてなる合成高分子を含んでなる請求項１～９のいずれか記載のポリペプチド含有基材。

【請求項１０】 請求項１～９のいずれかに記載のポリペプチド含有基材を用い、無血清培地にて動物細胞を培養することを特徴とする動物細胞の生産方法。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は、ポリペプチド含有基材に関する。さらに詳しくは、細胞の接着・増殖性が高く、特に、無血清培地を用いても、血清含有培地と同等以上の接着・増殖性を与える動物細胞培養用のポリペプチド含有基材に関するものである。

【０００２】

【従来の技術】Arg Gly Asp配列を有する遺伝子組換えペプチドであるプロネクチンＦ（以下、ＰｎＦと略する。）やポリ－Ｌ－リジンをコートした樹脂微粒子に対する細胞接着性を、０．５％の血清を含有した培地を使用して研究発表されている｛バラニ（J.Varani），インマン（D.R.Inman），フリギール（S.E.G.Fligiel），ヒリガス（W.J.Hillegas）、サイトテクノロジー（Cytotechnology）13,1993年、89-98頁）｝。

【０００３】

【発明が解決しようとする課題】この研究発表において、ＰｎＦを０．００５μｇ／ｃｍ²あるいは０．０２５μｇ／ｃｍ²コートした場合、コートしない場合のそれぞれ３倍又は５倍の細胞接着性を示すが、接着・増殖性がさらに高いものが強く要望されている。一方、同研究発表で、ポリ－Ｌ－リジンを０．５μｇ／ｃｍ²コートした場合に、コートしない場合の約４１倍の細胞接着性を示すが、この場合、細胞の伸展が遅いという問題がある。すなわち、本発明の目的は、動物細胞の接着性及び増殖性が高く、特に無血清培地でも動物細胞の接着性及び増殖性が高く、細胞を効率的に培養できる動物細胞培養用基材を提供することにある。

【０００４】

【課題を解決するための手段】本発明者は、鋭意研究を重ね、特定のポリペプチドと特定の官能基及び／又は特定の構造をその表面に配した基材を用いることにより、初期の細胞付着性が高まり、かつ、増殖性が高くなることを見いだし本発明に到達した。すなわち、本発明のポリペプチド含有基材の特徴は、細胞接着シグナルを現す最小アミノ酸配列を１分子中に少なくとも１個有するポリペプチド（Ｐ）を含んでなり、かつ２級アミノ基（Ａ１）、３級アミノ基（Ａ２）、アンモニオ基（Ａ３）、ホスファチジル基（Ａ４）及びリゾホスファチジル基（Ａ５）からなる群より選ばれる少なくとも１つの官能基（Ａ）、並びに／又は糖（Ｂ１）及びステロイド環（Ｂ２）から選ばれる少なくとも１つの構造（Ｂ）を有

してなる点を要旨とする。

【０００５】

【発明の実施の形態】細胞接着シグナルを現わす最小アミノ酸配列としては、接着シグナルとして働くものであればいずれも使用でき、例えば、株式会社永井出版発行「病態生理」Ｖｏｌ．９、Ｎｏ．７（１９９０）５２７頁に記載されているもの等が使用できる。

【０００６】これらのうち、接着する細胞の種類が多いという点で、Arg Gly Asp配列、LeuAsp Val配列、Arg Glu Asp Val配列（１）、Tyr Ile Gly Ser Arg配列（２）、Pro Asp Ser Gly Arg配列（３）、Arg Tyr Val Val Leu Pro Arg配列（４）、Leu Gly Thr Ile Pro Gly配列（５）、Arg Asn Ile Ala Glu Ile Ile Lys Asp Ile配列（６）、Ile Lys Val Ala Val配列（７）、Leu Arg Glu配列、Asp Gly GluAla配列（８）及びHis Ala Val配列が好ましく、さらに好ましくはArg Gly Asp配列、Ile Lys Val Ala Val配列（７）及びHis Ala Val配列である。これらの配列は１種又は２種以上を組み合わせて使用することができる。なお、アミノ酸配列はアミノ酸３文字表記で現わし、（　）内にアミノ酸配列表に対応する配列番号を付記した。

【０００７】ポリペプチド（Ｐ）中には前記最小アミノ酸配列が１分子中に少なくとも１個含有される必要がある。前記最小アミノ酸配列を含有すると、細胞接着活性が高まり、本来の機能を維持した状態で動物細胞の増殖をさらに促進することが可能となる。一方、前記最小アミノ酸配列が含有されない場合、細胞接着性が低下する。この結果、特に無血清培地を用いる場合に細胞の増殖が不十分になる。

【０００８】この最小アミノ酸配列の（Ｐ）１分子中の含有量は、細胞接着・増殖性の観点から、１分子中３～５０個が好ましく、さらに好ましくは５～４０個、特に好ましくは１０～３０個である。含有量がこの範囲であると、細胞接着活性がさらに高まり、本来の機能を維持した状態で動物細胞の増殖を促進することが容易となりやすい傾向にある。

【０００９】ポリペプチド（Ｐ）の数平均分子量（以下、Ｍｎと略する）は、細胞に対する毒性が低く、接着性能が高いという点で、５，０００以上が好ましく、さらに好ましくは１０，０００以上、特に好ましくは５０，０００以上である。また５，０００，０００以下が好ましく、さらに好ましくは１，０００，０００以下、特に好ましくは５００，０００以下である。なお、ポリペプチド（Ｐ）の（Ｍｎ）は、ＳＤＳ－ＰＡＧＥ法（Ｎａドデシルスルフェイト－ポリアクリルアミドゲル電気泳動法）で、（Ｐ）を水中で展開し、泳動距離を標準物質と比較することによって求められる。

【００１０】ポリペプチド（Ｐ）は、細胞接着シグナルを現わす最小アミノ酸配列以外に、（Ｐ）の熱安定性が高まるアミノ酸配列、例えばシルクフィブロイン由来のGlyAla Gly Ala Gly Ser配列（９）を少なくとも２個、分子中に有することが好ましく、このアミノ酸配列を５個以上有することがさらに好ましく、３～３０個有することが特に好ましい。

【００１１】ポリペプチド（Ｐ）としては、例えば、（Gly Ala Gly Ala Gly Ser）$_3$配列（１０）とArg Gly Asp配列とを有するポリペプチド、（Gly Ala Gly Ala Gly Ser）$_3$配列（１０）とTyr Ile Gly Ser Arg配列（２）とを有するポリペプチド、（Gly Ala Pro (Gly Pro Pro)$_4$）$_2$配列（１１）とArg Gly Asp配列とを有するポリペプチド、（Gly Ala Pro (Gly Pro Pro)$_4$）$_2$配列（１１）とTyr Ile Gly Ser Arg配列（２）とを有するポリペプチド、及び（Gly Ala Gly Ala Gly Ser）$_3$配列（１０）とIle Lys Val Ala Val配列（７）とを有するポリペプチド（特表平３－５０２９３５号公報）等が挙げられる。

【００１２】ポリペプチド（Ｐ）として市場から入手できるものとしては、例えば、三洋化成工業（株）製プロネクチンＦ（遺伝子組替大腸菌により製造され、１分子中にArg Gly Asp配列と（Gly Ala Gly Ala Gly Ser）$_3$配列（１０）とを各々約１３個有する（Ｍｎ）約１１万のポリペプチド）、同プロネクチンＦプラス（プロネクチンＦをジメルアミノエチルクロライドと反応させて水溶性にしたもの）、同プロネクチンＬ（遺伝子組替大腸菌により製造され、１分子中にIle Lys Val Ala Val配列（７）と（Gly Ala Gly Ala Gly Ser）$_3$配列（１０）とを各々約７個有する（Ｍｎ）約９万のポリペプチド）等が挙げられる。

【００１３】また、宝酒造（株）製ＲｅｔｒｏＮｅｃｔｉｎ（リコンビナントヒトフィブロネクチンＣＨ－２９６）｛ヒトフィブロネクチン細胞接着シグナルであるＣＳ１シグナルと細胞接着ドメインＴｙｐｅＩＩＩ及びヘパリン結合ドメインＩＩを１つずつ有する（Ｍｎ）約6万のポリペプチド｝、同ＲＧＤＳ－ＰｒｏｔｅｉｎＡ｛Arg Gly Asp配列をＰｒｏｔｅｉｎ Ａ（ＩｇＧ結合ドメイン）に挿入した（Ｍｎ）約３万のポリペプチド｝もポリペプチド（Ｐ）として使用可能である。

【００１４】ポリペプチド（Ｐ）の製造方法は特に制限されず、ペプチドを合成する従来既知の方法と同様にして製造することができ、例えば、有機合成法（固相合成法、液相合成法等）及び生化学的合成法［遺伝子組換微生物（酵母、細菌、大腸菌等）］等によって合成することができる。

【００１５】有機合成法に関しては、例えば、日本生化学学会編「続生化学実験講座２、タンパク質の化学（下）」第６４１～６９４頁（昭和６２年５月２０日；株式会社東京化学同人発行）に記載されている方法等が用いられる。

【００１６】生化学的合成法に関しては、例えば、特表平３－５０２９３５号公報に記載されている方法等が用

いられる。高分子量のポリペプチド（Ｐ）を容易に合成できる点で、遺伝子組換微生物による生化学的合成法が好ましく、特に好ましくは遺伝子組換大腸菌を用いて合成する方法である。

【００１７】ポリペプチド（Ｐ）は、その全て又は一部が基材表面に存在していることが好ましく、さらに好ましくは（Ｐ）の全てが基材表面に存在していることである。なお、基材表面とは、基材が容器状の場合、容器内に培養液を入れた場合に培養液が接し得る面を意味し、基材が粒子状の場合、基材を培養液中に入れた場合に培養液が接触し得る面を意味する。

【００１８】基材表面のポリペプチド（Ｐ）の量（μｇ／ｃｍ^2）としては、細胞の接着・増殖性の観点から、基材の表面積１ｃｍ^{2}当り０．1以上が好ましく、さらに好ましくは０．２以上、特に好ましくは０．３以上である。また経済性の観点から、１００以下が好ましく、さらに好ましくは５０以下、特に好ましくは１０以下である。なお、基材表面のポリペプチド（Ｐ）の量は、通常のタンパク量測定試薬（例えば、ピアスケミカル社製ＢＣＡ蛋白試薬等）で測定することができる。また、基材の表面積は、基材が容器状の場合は、この基材表面の形状から算出でき、基材が球状粒子の場合は、その平均粒子径及び密度から計算でき［粒子径；２ｒ（μｍ）、密度；ｄ（ｇ／ｃｍ^3）のとき、表面積（ｃｍ^2／ｇ）は、$4\pi r^2 / (4/3\pi r^3 \cdot d) = 3/(rd)$］、多孔質の粒子状の場合、ＢＥＴ値比表面積計（例えば、商品名：ＱＵＡＮＴＡＳＯＲＢ、ユアサアイオニクス社製、測定ガス：Ｈｅ／Ｋｒ＝９９．９／０．１体積%、検量ガス：窒素）で定量できる。

【００１９】２級アミノ基（Ａ１）としては、脂肪族アミノ基、芳香族アミノ基、－ＮＨＣＨ$_2$－で表される基及び－Ｃ（＝ＮＨ）－で表される基等が使用できる。これらの炭素数は１～１６が好ましく、さらに好ましくは１～１２、特に好ましくは１～６である。

【００２０】脂肪族アミノ基としては、メチルアミノ基、エチルアミノ基、プロピルアミノ基、ブチルアミノ基、シクロヘキシルアミノ基、２－エチルヘキシルアミノ基、ドデシルアミノ基、ヘキサデシルアミノ基、ベンジルアミノ基及び２－フェニルエチルアミノ基等が挙げられる。

【００２１】芳香族アミノ基としては、フェニルアミノ基（アニリノ基）、４－メチルフェニルアミノ基（４－メチルアニリノ基）及びナフチルアミノ基等が挙げられる。これらのうち、脂肪族アミノ基、－ＮＨＣＨ$_2$－で表される基及び－Ｃ（＝ＮＨ）－で表される基が好ましく、さらに好ましくはメチルアミノ基、エチルアミノ基、プロピルアミノ基、－ＮＨＣＨ$_2$－で表される基及び－Ｃ（＝ＮＨ）－で表される基、特に好ましくは－ＮＨＣＨ$_2$－で表される基及び－Ｃ（＝ＮＨ）－で表される基である。

【００２２】３級アミノ基（Ａ２）としては、脂肪族アミノ基、芳香族アミノ基、－Ｎ（ＣＨ$_2$－ＯＨ）ＣＨ$_2$－又は－Ｎ（ＣＨ$_3$）ＣＨ$_2$－で表される基等が使用できる。これらの炭素数は２～３２が好ましく、さらに好ましくは２～２４、特に好ましくは２～１２である。

【００２３】脂肪族アミノ基としては、ジメチルアミノ基、ジエチルアミノ基、メチルプロピルアミノ基、ジイソプロピルアミノ基、ジブチルアミノ基、ジシクロヘキシルアミノ基、ジドデシルアミノ基、ジヘキサデシルアミノ基及びジベンジルアミノ基等が挙げられる。

【００２４】芳香族アミノ基としては、ジフェニルアミノ基及びジナフチルアミノ基等が挙げられる。　これらのうち、脂肪族アミノ基、－Ｎ（ＣＨ$_2$－ＯＨ）ＣＨ$_2$－又は－Ｎ（ＣＨ$_3$）ＣＨ$_2$－で表される基が好ましく、さらに好ましくは、ジメチルアミノ基、ジエチルアミノ基、ジプロピルアミノ基及び－Ｎ（ＣＨ$_2$－ＯＨ）ＣＨ$_2$－又は－Ｎ（ＣＨ$_3$）ＣＨ$_2$－で表される基、特に好ましくはジメチルアミノ基、ジエチルアミノ基及び－Ｎ（ＣＨ$_2$－ＯＨ）ＣＨ$_2$－又は－Ｎ（ＣＨ$_3$）ＣＨ$_2$－で表される基である。

【００２５】アンモニオ基（Ａ３）としては、脂肪族アンモニオ基、芳香族アンモニオ基、＞Ｃ＝Ｎ$^+$＝Ｃ＜で表される基及び－ＣＨ$_2$Ｎ（ＣＨ$_3$）$_2$ＣＨ$_2$－で表される基等が使用できる。これらの炭素数は１～４８が好ましく、さらに好ましくは２～３６、特に好ましくは２～１８である。

【００２６】脂肪族アンモニオ基としては、メチルアンモニオ基、エチルアンモニオ基、プロピルアンモニオ基、ブチルアンモニオ基、シクロヘキシルアンモニオ基、２－エチルヘキシルアンモニオ基、ドデシルアンモニオ基、ヘキサデシルアンモニオ基、ベンジルアンモニオ基、２－フェニルエチルアンモニオ基、ジメチルアンモニオ基、ジエチルアンモニオ基、メチルプロピルアンモニオ基、ジイソプロピルアンモニオ基、ジブチルアンモニオ基、ジシクロヘキシルアンモニオ基、ジドデシルアンモニオ基、ジヘキサデシルアンモニオ基、ジベンジルアンモニオ基、トリメチルアンモニオ基、トリエチルアンモニオ基、メチルジエチルアンモニオ基、メチルジイソプロピルアンモニオ基、メチルジブチルアンモニオ基、メチルジシクロヘキシルアンモニオ基、メチルジドデシルアンモニオ基、トリヘキサデシルアンモニオ基及びトリベンジルアンモニオ基等が挙げられる。

【００２７】芳香族アンモニオ基としては、ベンジルアンモニオ基、メチルフェニルアンモニオ基、メチルナフチルアンモニオ基、メチルジフェニルアミノ基、メチルジナフチルアミノ基及びトリベンジルアンモニオ基等が挙げられる。

【００２８】これらのうち、脂肪族アンモニオ基、＞Ｃ＝Ｎ$^+$＝Ｃ＜で表される基及び－ＣＨ$_2$Ｎ（ＣＨ$_3$）$_2$ＣＨ$_2$－で表される基が好ましく、さらに好ましくは、メチ

ルアンモニオ基、エチルアンモニオ基、プロピルアンモニオ基、ブチルアンモニオ基、トリメチルアンモニオ基、トリエチルアンモニオ基、メチルジエチルアンモニオ基、メチルジイソプロピルアンモニオ基、メチルジブチルアンモニオ基、＞Ｃ＝Ｎ⁺＝Ｃ＜で表される基及び－$CH_2N(CH_3)_2CH_2$－で表される基、特に好ましくは、メチルアンモニオ基、エチルアンモニオ基、トリメチルアンモニオ基、トリエチルアンモニオ基、メチルジエチルアンモニオ基、＞Ｃ＝Ｎ⁺＝Ｃ＜で表される基及び－$CH_2N(CH_3)_2CH_2$－で表される基である。

【００２９】ホスファチジル基（Ａ４）としては、$HOCH_2CH(OH)CH_2OP(=O)(O^-)$－で表される基（無置換ホスファチジル基）又は$R^1OCH_2CH(OR^2)CH_2OP(=O)(O^-)$－で表される基（置換ホスファチジル基、$R^1$及び$R^2$は、同じ又は異なる飽和又は不飽和のアシル基、アルキル基又はアルケニル基である）等が使用できる。アシル基、アルキル基又はアルケニル基の炭素数は１～２２が好ましく、さらに好ましくは２～１８、特に好ましくは１２～１８である。

【００３０】アシル基としては、飽和アシル基（アセチル基、ブタノイル基、ヘキサノイル基、デカノイル基、ラウロイル基、ミリストイル基、パルミトイル基、ステアロイル基及びアラキドノイル基等）、及び不飽和アシル基（リノレオイル基、オクタデシエノイル基及びオレオイル基等）等が挙げられる。

【００３１】アルキル基としては、メチル基、ヘキサデシル基、ブチル基、ヘキキル基、デシル基、ラウリル基、ミリスチル基、パルミチル基、ステアリル基及びアラキドイル基等が挙げられる。

【００３２】アルケニル基としては、リノレイル基、オクタデシルジエニル基及びオレオイル基等が挙げられる。これらのうち、無置換ホスファチジル基及び置換ホスファチジル基（R^1及びR^2はアシル基である）が好ましく、さらに好ましくは無置換ホスファチジル基及び置換ホスファチジル基（R^1及びR^2は不飽和アシル基である）、特に好ましくは置換ホスファチジル基（R^1及びR^2は同じ不飽和アシル基である）ホスファチジル基である。

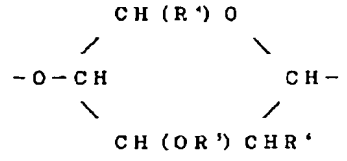
【００３３】リゾホスファチジル基（Ａ５）としては、$R^3OCH_2CH(OH)CH_2OP(=O)(O^-)$－で表される基（$R^3$は、飽和又は不飽和のアシル基）等が使用できる。アシル基の炭素数は１～２２が好ましく、さらに好ましくは２～１８、特に好ましくは１２～１８である。

【００３４】アシル基としては、ホスファチジル基（Ａ４）の場合と同じものが使用できる。これらのうち、R^3が不飽和アシル基である置換リゾホスファチジル基が好ましい。

【００３５】糖（Ｂ１）としては、次の化学式（化１）で表される基を１個以上有する構造等が使用でき、例えば、天然又は合成の糖が含有する構造等が用いられる。

【００３６】

【化１】



【００３７】（R^4は、－Ｈ、$-COO^-$、$-CH_2OH$、$-CH_2OCOCH_3$、$-CH_2OSO_3^-$又は$-CH_3$のいずれかを表し、R^5は、－Ｈ、$-COCH_3$、$-CH_3$又は$-CH(CH_3)COO^-$のいずれかを表し、R^6は、－ＯＨ、$-OCOCH_3$、$-NH_2$、$-NHCOCH_3$、$-NHCOCH_2OH$又は－Ｆのいずれかを表す。）

【００３８】天然又は合成の糖としては、単糖類（ガラクトース、グルコース、マンノース、フコース、グルコサミン、ガラクトサミン、Ｎ－アセチルガラクトサミン、Ｎ－メチルグルコサミン、Ｎ－アセチルニューラミン酸、アラビノース、デオキシグルコース、デオキシフルオログルコース、リボース、デオキシリボース、エリスロース、フルクトース、イノシトール、リクソース、マドゥロース、ムラミン酸、デオキシマンオース、ソルボース、キシロース及びトリアセチルグルカール等）、多糖類（セロビオース、ジフルクトース、コジビオース、ラクトース、ラクツロース、マルチトール、マルトース、トレハロース、ガラクトシラクトース、グルコシルスクロース、イソマルトオリゴサッカライド、マルトオリゴサッカライド、テハロサミン、パノース、キシロオリゴサッカロースフフルクトオリゴサッカライド、ニストース、スタキロース、キトオリゴ糖、キトサンオリゴ糖、ムコ多糖、アルギニン酸、カードラン、デキストラン、レバン、パラミオール、ポリデキストロース、プルラン、スターチ及びシクロデキストリン等）等が挙げられる。

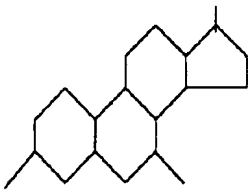
【００３９】これらのうち、ガラクトース、グルコース、マンノース、フコース、グルコサミン、ガラクトサミン、Ｎ－メチルグルコサミン、Ｎ－アセチルガラクトサミン及びＮ－アセチルニューラミン酸が好ましく、さらに好ましくはガラクトース、グルコース、マンノース、フコース、Ｎ－アセチルガラクトサミン及びＮ－アセチルニューラミン酸である。

【００４０】ステロイド環（Ｂ２）としては、次の化学式（化２）で表される環構造等が使用でき、例えば、天然又は合成の胆汁酸又はステロールに含まれるステロイド環等が用いられる。

【００４１】

【化２】

9



【００４２】天然又は合成の胆汁酸としては、ケノジオール、コール酸、デオキシコール酸、グリココール酸、グリコリトコール酸、リソコール酸、スクアレン及びタウロコール酸等が挙げられる。

【００４３】天然又は合成のステロールとしては、ブラシカステロール、カンペステロール、コレステロール、エルゴステロール、フコステロール、ラノステロール、シトステロール及びスチグマステロール等が挙げられる。これらのうち、胆汁酸に含まれるステロイド環が好ましく、さらに好ましくはコール酸、デオキシコール酸、グリココール酸、グリコリトコール酸又はリソコール酸に含まれるステロイド環である。

【００４４】官能基（Ａ）及び構造（Ｂ）は、その全て又は一部が基材表面に存在していることが好ましく、全てが基材表面に存在していることがさらに好ましい。基材表面の官能基（Ａ）及び構造（Ｂ）の含有量（個／ｃｍ²）は、細胞の初期付着性向上効果又は増殖性向上効果の観点から、基材の表面積１ｃｍ²当り、１×１０¹³～1×１０²⁰が好ましく、さらに好ましくは１×１０¹⁵～１×１０¹⁹、特に好ましくは１×１０¹⁵～１×１０¹⁷である。

【００４５】基材表面の官能基（Ａ）及び構造（Ｂ）の含有量は、官能基（Ａ）及び構造（Ｂ）の種類によって以下の方法により定量される。２級アミノ基（Ａ１）、３級アミノ基（Ａ２）及びアンモニオ基（Ａ３）の場合、通常のアミン価測定試薬（例えば、塩酸水溶液又は硝酸銀水溶液等）を用いて、電位差滴定によりアミン価を測定することにより求めることができる。

【００４６】ホスファチジル基（Ａ４）、リゾホスファチジル基（Ａ５）、糖（Ｂ１）及びステロイド環（Ｂ２）の場合、酵素定量法により求めることができる。すなわち、ホスファチジル基（Ａ４）及びリゾホスファチジル基（Ａ５）の場合、例えば、カイノス社製リン脂質測定キット「アクアオート　カイノス　ＰＬ試薬」等を用いることにより求めることができる。また、糖（Ｂ１）の場合、ロッシュダイアグノスティックス社製「Ｆ－キット　糖質用」を用いることにより求めることができる。また、ステロイド環（Ｂ２）の場合、例えば、ロッシュダイアグノスティックス社製「Ｆ－キット　ステロイド用」等を用いることにより求めることができる。

【００４７】本発明のポリペプチド含有基材の製造に用いられる基材としては、細胞培養用基材として通常使用されるもの等が使用でき、例えば、シャーレ、プレート、フラスコ、ローラーボトル、マイクロキャリアビーズ及びホローファイバー等が挙げられる。これらの基材の素材としては、無機物（ガラス、セラミックス及びヒドロキシアパタイト等）、及び有機物｛合成高分子［ビニル樹脂（ポリスチレン、ポリメチルメタクリレート、ポリビニルホルマール、ポリメチルメンテン、ポリビニルアルコール、ポリアクリルアミド、ポリエチレン及びポリプロピレ等）、ポリエステル、ポリカーボネート、ウレタン樹脂及びエポキシ樹脂］及び天然高分子（ポリデキスラン、セルロース、コラーゲン及びゼラチン等）｝等が挙げられる。

【００４８】シャーレとしては、基材の表面積（培養面積）が５～５００ｃｍ²／個のものが挙げられる。プレートとしては、２～３８４穴／プレートのものが挙げられる。フラスコとしいては、基材の表面積（培養面積）が１０～５００ｃｍ²／個のＴ－フラスコ及びスピナーフラスコ等が挙げられる。ローラーボトルとしては、容量が０．１～１０Ｌ／個のもの等が挙げられる。マイクロキャリアビーズとしては、粒子径；２０～５００μｍ、密度；１．０～１．１ｇ／ｃｍ³、基材の表面積（培養面積）；１００～１００，０００ｃｍ²／ｇのものが挙げられる。ホローファイバーとしては、内径が１０～５００μｍのもの等が挙げられる。

【００４９】これらの基材のうち、効率的に高密度の細胞が得られるという点で、マイクロキャリアビーズが好ましい。粒子径（μｍ）としては、単位重量当りの表面積が大きく、また、細胞が接着し易いという点で、３０以上が好ましく、さらに好ましくは４０以上、特に好ましくは５０以上である。また３００以下が好ましく、さらに好ましくは２００以下、特に好ましくは１００以下である。また、密度（ｇ／ｃｍ³）としては、緩やかな撹拌で浮遊し、また、撹拌を停止すると速やかに沈降しやすいという点で、１．０以上が好ましく、さらに好ましくは１．０１以上、特に好ましくは１．０２以上である。また１．１以下が好ましく、さらに好ましくは１．０５以下、特に好ましくは１．０４以下である。

【００５０】また、細胞との親和性が高く、耐熱性に優れるという点で、スチレン及び多官能性モノマー［ジビニルベンゼン、エチレングリコールジ（メタ）アクリレート、トリビニルベンゼン及びトリメチロールプロパントリ（メタ）アクリレート等］を必須構成単量体としてなる合成高分子を含有するものが好ましい。この場合の多官能モノマーの含有量（重量％）は、基材の重量に基づいて、０．１以上が好ましく、さらに好ましくは０．５以上、特に好ましくは１以上である。また４０以下が好ましく、さらに好ましくは２０以下、特に好ましくは１０以下である。

【００５１】本発明のポリペプチド含有基材は、官能基（Ａ）及び／又は構造（Ｂ）を有する基材にポリペプチド（Ｐ）を配することにより（例えばコーティングにより）、基材に（Ｐ）を配した後官能基（Ａ）及び／又は

構造（Ｂ）を有する化合物を配することにより（例えばコーティングにより）、又は、基材に官能基（Ａ）及び／又は構造（Ｂ）を有する化合物と（Ｐ）を同時に配することにより製造することができる。

【００５２】ポリペプチド（Ｐ）を基材（の表面）に配する方法としては、例えば、ポリペプチド（Ｐ）を溶媒に溶かした溶液又は分散させた分散液を予め作製し、これと基材を接触させた後、乾燥する方法等が適用できる。

【００５３】ポリペプチド（Ｐ）の溶液又は分散液を作製するために用いられる溶媒としては特に制限はないが、無機塩、有機酸塩、アミノ酸、ビタミン、アルコール、脂質・糖、酸及び／又は塩基を含有する水溶液及び水等が使用できる。

【００５４】無機塩としては、ハロゲン化金属塩、硫酸金属塩、リン酸金属塩、硝酸金属塩、炭酸金属塩及び過ハロゲン酸金属等が使用でき、例えば、塩化ナトリウム、塩化カルシウム、塩化カリウム、塩化リチウム、臭化ナトリウム、臭化リチウム、硫酸ナトリウム、硫酸マグネシウム、硫酸銅、硫酸鉄、リン酸ナトリウム、リン酸水素ナトリウム、リン酸カリウム、リン酸水素カリウム、硝酸鉄、炭酸ナトリウム、過塩素酸ナトリウム及び過塩素酸リチウム等が挙げられる。有機酸塩としては、炭素数１～４の有機酸金属塩等が使用でき、例えば、蟻酸ナトリウム、酢酸ナトリウム、酢酸リチウム及び酒石酸ナトリウム等が挙げられる。

【００５５】アミノ酸としては、天然アミノ酸等が使用でき、例えば、アルギニン、ヒスチジン、イソロイシン、ロイシン、メチオニン、フェニルアラニン、スレオニン、トリプトファン、チロシン、バリン、アラニン、アスパラギン、アスパラギン酸、グルタミン酸、プロリン、セリン及びグリシン等が挙げられる。ビタミンとしては、例えば、コリン、イノシトール、ニコチンアミド、グルタミン、ビタミンＡ及びビタミンB_{12}等が挙げられる。アルコールとしては、炭素数１～４のアルコール等が使用でき、例えば、メタノール、エタノール、イソプロピルアルコール及びブタノール等が挙げられる。

【００５６】脂質・糖としては、天然糖等が使用でき、例えば、脂質、単糖、２糖、オリゴ糖、アミノ糖及び酸性糖等が挙げられる。酸としては、無機酸及び炭素数１～６の有機酸等が使用でき、例えば、塩酸、硝酸、硫酸、燐酸、酢酸、蟻酸、酒石酸、リンゴ酸、メタンスルホン酸、フェノール及びカテコール等が挙げられる。塩基としては、無機塩基及び炭素数２～６の有機塩基等が使用でき、例えば、水酸化ナトリウム、水酸化カリウム、炭酸水素ナトリウム、アンモニア、モノエタノールアミン、トリエタノールアミン及びトリエチルアミン等が挙げられる。

【００５７】水としては、蒸留水、イオン交換水、水道水及びイオン交換蒸留水等が挙げられる。これらの溶媒の中で、無機塩、酸及び／又は塩基を含有する水溶液並びに水が好ましく、さらに好ましくは無機塩を含有する水溶液及び水、特に好ましくは無機塩を含有する水溶液である。なお、これらの含有量（重量%）としては、水溶液又は分散液の重量に基づいて、０．１以上が好ましく、さらに好ましくは１以上である。また５０以下が好ましく、さらに好ましくは３０以下である。

【００５８】ポリペプチド（Ｐ）の溶液又は分散液中の（Ｐ）の濃度は、溶媒１ｍｌ当り、０．０１μｇ以上が好ましく、さらに好ましくは０．１μｇ以上、特に好ましくは１μｇ以上である。また１００ｍｇ以下が好ましく、さらに好ましくは１０ｍｇ以下、特に好ましくは１ｍｇ以下である。ペプチド（Ｐ）の溶液又は分散液と基材との接触は、溶液又は分散液を基材に振りかける方法、溶液又は分散液に基材を浸漬する方法等のいずれでもよい。

【００５９】接触時間としては、用いる基材によっても異なるが、３０秒以上が好ましく、さらに好ましくは１分以上、特に好ましくは３分以上である。また４８時間以下が好ましく、さらに好ましくは２４時間以下、特に好ましくは１２時間以下である。必要に応じて行われる乾燥の条件についても特に制限はなく、通常の方法が適用でき、例えば、必要に応じて順風乾燥機や減圧乾燥機などを用いて、０～２００℃、０．００１Ｐａ～大気圧の圧力下で、１～１００時間乾燥することで行える。

【００６０】また、必要に応じて行われる乾燥の前又は後で、無機塩を含有する水溶液又は水で通常の方法で洗浄することもできる。また、接触の後で、必要に応じて滅菌処理を施してもよい。滅菌方法は特に制限は無く、例えば、放射線滅菌、エチレンオキサイドガス滅菌、オートクレーブ滅菌及び乾熱滅菌等が挙げられる。

【００６１】官能基（Ａ）及び／又は構造（Ｂ）を基材に配する方法としては、①ビニル樹脂を主成分とする基材の場合、（Ａ）及び/又は（Ｂ）を有するモノマーを、ビニル樹脂を主成分とする基材を作成する時に共重合する方法、②（Ａ）及び／又は（Ｂ）を有する反応性化合物を基材に共有結合させる方法、③（Ａ）及び/又は（Ｂ）を有する化合物を基材の表面に接触させる方法、④（Ａ）及び/又は（Ｂ）を有する化合物を基材を作製する時に基材を構成する材料に混合する方法等が挙げられる。

【００６２】なお、（Ａ）及び／又は（Ｂ）は、基材表面又はポリペプチド（Ｐ）等に化学結合していても、（Ａ）及び／又は（Ｂ）を有する化合物として基材表面又は（Ｐ）等に物理吸着してもよい。糖が化学結合する場合、糖の１，３，４位のいずれかで化学結合させること、ステロイドが化学結合する場合、ステロイドの３，７，１４位のいずれかで結合させることができる。

【００６３】①の方法において、官能基（Ａ）及び／又は構造（Ｂ）を有するモノマーとしては、２級アミノ基

（Ａ１）を有するモノマー（ｍａ１）、３級アミノ基（Ａ２）を有するモノマー（ｍａ２）、アンモニオ基（Ａ３）を有するモノマー（ｍａ３）、ホスファチジル基（Ａ４）を有するモノマー（ｍａ４）、リゾホスファチジル基（Ａ５）を有するモノマー（ｍａ５）、糖（Ｂ１）を有するモノマー（ｍｂ１）、ステロイド環（Ｂ２）を有するモノマー（ｍｂ２）及びこれらの２種以上の混合物等が使用できる。

【００６４】２級アミノ基（Ａ１）を有するモノマー（ｍａ１）としては、２級アミノ基含有（メタ）アクリレート［Ｎ－メチルアミノエチル（メタ）アクリレート、Ｎ－エチルアミノプロピル（メタ）アクリレート、Ｎ－プロピルアミノエチル（メタ）アクリレート、Ｎ－ベンジルアミノエチル（メタ）アクリレート及びピペリジノエチル（メタ）アクリレート等］、２級アミノ基含有（メタ）アクリルアミド［Ｎ－メチルアミノエチル（メタ）アクリルアミド、Ｎ－エチルアミノプロピル（メタ）アクリルアミド、Ｎ－プロピルアミノエチル（メタ）アクリルアミド及びＮ－ベンジルアミノエチル（メタ）アクリルアミド等］、２級アミノ基含有芳香族ビニル炭化水素（Ｎ－メチルアミノスチレン、Ｎ－プロピルアミノスチレン及びＮ－ベンジルアミノスチレン等）及び２級アミノ基含有アリルエーテル（Ｎ－メチルアミノエチルアリルエーテル及びＮ－エチルアミノエチルアリルエーテル等）等が挙げられる。

【００６５】３級アミノ基（Ａ２）を有するモノマー（ｍａ２）としては、３級アミノ基含有（メタ）アクリレート［Ｎ，Ｎ－ジメチルアミノエチル（メタ）アクリレート、Ｎ，Ｎ－ジエチルアミノプロピル（メタ）アクリレート、Ｎ，Ｎ－ジプロピルアミノエチル（メタ）アクリレート、Ｎ－ベンジル－Ｎ－メチルアミノエチル（メタ）アクリレート、モルホリノエチル（メタ）アクリレート及びＮ－メチルピペチジノエチル（メタ）アクリレート等］、３級アミノ基含有（メタ）アクリルアミド［Ｎ，Ｎ－ジメチルアミノエチル（メタ）アクリルアミド、Ｎ，Ｎ－ジエチルアミノプロピル（メタ）アクリルアミド、Ｎ，Ｎ－ジプロピルアミノエチル（メタ）アクリルアミド、Ｎ－ベンジル－Ｎ－メチルアミノエチル（メタ）アクリルアミド、モルホリノエチル（メタ）アクリルアミド及びＮ－メチルピペチジノエチル（メタ）アクリルアミド等］、３級アミノ基含有芳香族ビニル炭化水素（Ｎ，Ｎ－ジメチルアミノスチレン、Ｎ，Ｎ－ジプロピルアミノスチレン及びＮ－ベンジル－Ｎ－メチルアミノスチレン等）及び３級アミノ基含有アリルエーテル（Ｎ，Ｎ－ジメチルアミノエチルアリルエーテル及びＮ，Ｎ－ジエチルアミノエチルアリルエーテル等）等が挙げられる。

【００６６】アンモニオ基（Ａ３）を有するモノマー（ｍａ３）としては、アンモニオ基含有（メタ）アクリレート［（メタ）アクリロイルオキシエチルアンモニウム・クロリド、メチル　（メタ）アクリロイルオキシエチルアンモニウム・クロリド、ジメチル　（メタ）アクリロイルオキシエチルアンモニウム・クロリド、トリメチル　（メタ）アクリロイルオキシエチルアンモニウム・クロリド及びジエチルメチル　（メタ）アクリロイルオキシエチルアンモニウム・クロリド等］、アンモニオ基含有（メタ）アクリルアミド［（メタ）アクリロイルアミノエチルアンモニウム・クロリド、メチル　（メタ）アクリロイルアミノエチルアンモニウム・クロリド、ジメチル　（メタ）アクリロイルアミノエチルアンモニウム・クロリド、トリメチル　（メタ）アクリロイルアミノエチルアンモニウム・クロリド及びジエチルメチル　（メタ）アクリロイルアミノエチルアンモニウム・クロリド等］、アンモニオ基含有芳香族ビニル炭化水素（トリメチル　ビニルフェニルアンモニウム・クロリド等）及びアンモニオ基含有アリルエーテル（トリエチル　アリルオキシエチルアンモニウム・クロリド等）等が挙げられる。

【００６７】ホスファチジル基（Ａ４）を有するモノマー（ｍａ４）としては、ホスファチジル基含有（メタ）アクリレート［ホスファチジルエチル（メタ）アクリレート、ジパルミトイルホスファチジルエチル（メタ）アクリレート及びジアラキドノイルホスファチジルプロピル（メタ）アクリレート等］、ホスファチジル基含有（メタ）アクリルアミド［ホスファチジルエチル（メタ）アクリレート、ジパルミトイルホスファチジルエチル（メタ）アクリレート及びジアラキドノイルホスファチジルプロピル（メタ）アクリレート等］、ホスファチジル基含有芳香族ビニル炭化水素（ジパルミトイルホスファチジルスチレン等）及びホスファチジル基含有アリルエーテル（ジパルミトイルホスファチジルエチルアリルエーテル等）等が挙げられる。

【００６８】リゾホスファチジル基（Ａ５）を有するモノマー（ｍａ４）としては、リゾフォスファチジル基含有（メタ）アクリレート［パルミトイルジゾホスファチジルエチル（メタ）アクリレート及びアラキドノイルリゾホスファチジルプロピル（メタ）アクリレート等］；リゾフォスファチジル基含有（メタ）アクリルアミド［パルミトイルリゾホスファチジルエチル（メタ）アクリレート及びアラキドノイルリゾホスファチジルプロピル（メタ）アクリレート等］、リゾフォスファチジル基含有芳香族ビニル炭化水素（パルミトイルリゾホスファチジルスチレン等）及びリゾフォスファチジル基含有アリルエーテル（パルミトイルリゾホスファチジルエチルアリルエーテル等）等が挙げられる。糖（Ｂ１）を有するモノマー（ｍｂ１）としては、糖含有芳香族ビニル炭化水素（Ｎ－ｐ－ビニルベンジルラクトノアミド等）及び糖含有（メタ）アクリレート［グルコース－６－（メタ）アクリレート等］等が挙げられる。

【００６９】ステロイド環（Ｂ２）を有するモノマー

（ｍｂ２）としては、ステロイド環含有（メタ）アクリレート［コレステロール（メタ）アクリレート及びエルゴステロール（メタ）アクリレート等］が挙げられる。これらのモノマーを用いて共重合する方法としては、ビニル樹脂を構成するモノマーにこれらを配合して、通常の方法（バルク重合、懸濁重合等）で共重合させる方法等が挙げられる。共重合させるときのこれらモノマーの含有量（重量%）は、構成モノマーの全重量に基づいて、０．００１以上が好ましく、さらに好ましくは０．１以上である。また、３０以下が好ましく、さらに好ましくは２０以下である。

【００７０】②の方法において、官能基（Ａ）及び／又は構造（Ｂ）を有する反応性化合物としては、２級アミノ基（Ａ１）を有する反応性化合物、３級アミノ基（Ａ２）を有する反応性化合物、アンモニオ基（Ａ３）を有する反応性化合物、ホスファチジル基（Ａ４）を有する反応性化合物、リゾホスファチジル基（Ａ５）を有する反応性化合物、糖（Ｂ１）を有する反応性化合物、ステロイド環（Ｂ２）を有する反応性化合物及びこれらの２種以上の混合物等が使用できる。２級アミノ基（Ａ１）を有する反応性化合物としては、上記の（Ａ１）を有するモノマーのほか、炭素数３～５の２級アミノ基含有ハロゲン炭化水素（メチルアミノエチルクロリド及びメチルアミノプロピルクロリド等）、炭素数４～８の２級アミノ基含有エポキシド（メチルアミノプロピルエポキシド、メチルアミノブチルエポキシド及びメチルアミノエチルグリシジルエーテル等）等が挙げられる。

【００７１】３級アミノ基（Ａ２）を有する反応性化合物としては、上記の（Ａ２）を有するモノマーのほか、炭素数４～６の３級アミノ基含有ハロゲン炭化水素（ジメチルアミノエチルクロリド及びジエチルアミノエチルクロリド等）、炭素数５～８の３級アミノ基含有エポキシド（ジメチルアミノプロピルエポキシド、ジメチルアミノエチルグリシジルエーテル等）等が挙げられる。

【００７２】アンモニオ基（Ａ３）を有する反応性化合物としては、上記の（Ａ３）を有するモノマーのほか、炭素数５～８のアンモニオ基含有ハロゲン炭化水素（トリメチルクロロエチルアンモニウムクロリド等）、炭素数６～１０のアンモニオ基含有エポキシド（トリメチルグリシジルエチルアンモニウムクロリド等）等が挙げられる。

【００７３】ホスファチジル基（Ａ４）を有する反応性化合物としては、上記の（Ａ４）を有するモノマーのほか、炭素数２～６のホスファチジル基含有ハロゲン炭化水素（ホスファチジルエチルクロリド及びジミリストイルホスファチジルエチルクロリド等）、炭素数３～８のホスファチジル基含有エポキシド（ホスファチジルエチルグリシジルエーテル及びジミリストイルホスファチジルエチルグリシジルエーテル等）等が挙げられる。

【００７４】リゾホスファチジル基（Ａ５）を有する反応性化合物としては、上記の（Ａ５）を有するモノマーのほか、炭素数２～６のリゾフォスファチジル基含有ハロゲン炭化水素（ミリストイルリゾホスファチジルエチルクロリド等）、炭素数３～８のリゾホスファチジル基含有エポキシド（ミリストイルリゾホスファチジルエチルグリシジルエーテル等）等が挙げられる。

【００７５】糖（Ｂ１）を有する反応性化合物としては、上記の（Ｂ１）を有するモノマーのほか、炭素数６～１８の糖含有ハロゲン炭化水素（グルコース－６－エチルクロリド等）、炭素数６～１８の糖含有エポキシド（グルコース－６－グリシジルエーテル等）等が挙げられる。

【００７６】ステロイド環（Ｂ２）を有する反応性化合物としては、上記の（Ｂ２）を有するモノマーのほか、炭素数１７～３２のステロイド環含有ハロゲン化物（クロロエチルコレステロール等）、炭素数１７～３２のステロイド環含有エポキシド（グリシジルコレステロール等）等が挙げられる。

【００７７】基材とこれらの反応性化合物を共有結合させるとき、上記のモノマーを用いる場合は、パーオキシド（過塩素酸ナトリウム、ジブチルパーオキシド、ジクミルパーオキシド、クメンハイドロパーオキシド等）とともに、基材とこれらの反応性化合物（水懸濁液としてもよい）を窒素雰囲気下、５０～１２０℃で１～４８時間反応させる方法を適用してもよい。

【００７８】また、ハロゲン炭化水素又はエポキシドを用いる場合、塩基（水酸化ナトリウム、水酸化カリウム、トリエチルアミン、１，８－ジアザビシクロ［５，４，０］ウンデセン－７（登録商標：ＤＢＵ）等）とともに、基材とこれらのハロゲン炭化水素又はエポキシド（水懸濁液としてもよい）を、２０～９０℃で１～４８時間反応させる方法等を適用してもよい。反応性化合物の使用量（ｇ／ｃｍ²）は、基材の表面積１ｃｍ²当り、1×10^{-8}以上が好ましく、さらに好ましくは1×10^{-6}以上²である。また1以下が好ましく、さらに好ましくは1×10^{-2}以下である。

【００７９】③の方法において、官能基（Ａ）及び／又は構造（Ｂ）を有する化合物としては、２級アミノ基（Ａ１）を有する化合物、３級アミノ基（Ａ２）を有する化合物、アンモニオ基（Ａ３）を有する化合物、ホスファチジル基（Ａ４）を有する化合物、リゾホスファチジル基（Ａ５）を有する化合物、糖（Ｂ１）を有する化合物、ステロイド環（Ｂ２）を有する化合物及びこれらの２種以上の混合物等が使用できる。

【００８０】２級アミノ基（Ａ１）を有する化合物としては、上記の（Ａ１）を有するモノマーからなる（共）重合体のほか、重量平均分子量（以下Ｍｗと略する）５００～１，０００，０００の２級アミノ基含有ポリマー（例えば、ポリエチレンイミン、ジシアンジアミド・ホルマリン縮合物、ジシアンジアミド・ジエチレントリア

ミン重縮合物、ジシアンジアミド・ジエチレントリアミン・尿素重縮合物及びジアリルアミン塩・二酸化硫黄共重合物等）等が挙げられる。なお、Ｍｗは、ポリエチレングリコールを基準物質としてゲルパーミエーションクロマトグラフィーにより測定されるものである。

【００８１】３級アミノ基（Ａ２）を有する化合物としては、上記の（Ａ２）を有するモノマーからなる（共）重合体のほか、Ｍｗ５００～１，０００，０００の３級アミノ基含有ポリマー（ジシアンジアミド・ジエチレントリアミン重縮合物、ジシアンジアミド・ジエチレントリアミン・尿素重縮合物、ポリヒスチジン及びポリ（Ｎ－メチルエチレンイミン）等）等が挙げられる。

【００８２】アンモニオ基（Ａ３）を有する化合物としては、上記の（Ａ３）を有するモノマーからなる（共）重合体のほか、Ｍｗ５００～１，０００，０００のアンモニオ基含有ポリマー（ポリエチレンイミンのメチルクロリド４級化物、ジシアンジアミド・ホルマリン縮合物、エピクロルヒドリン・ジメチルアミン付加重合物、ジメチルジアリルアンモニウムクロリド・二酸化硫黄共重合物、ジメチルジアリルアンモニウムクロリド重合物等）等が挙げられる。

【００８３】ホスファチジル基（Ａ４）を有する化合物としては、上記の（Ａ４）を有するモノマーからなる（共）重合体のほか、ホスファチジル基含有化合物｛ホスファチジン酸（鶏卵黄身由来のホスファチジン酸ナトリウム、β－アラキドノイル－γ－ステアロイルホスファチジン酸ナトリウム、ホスファチジン酸、ジラウロイルホスファチジン酸、ジオレイルホスファチジン酸、ジラウロイルホスファチジン酸ナトリウム及びジオレオイルホスファチジン酸ナトリウム等）、ホスファチジルコリン（鶏卵黄身由来ホスファチジルコリンの水素添加物、β－アセチル－γ－ヘキサデシルホスファチジルコリン、ジアラキドイルホスファチジルコリン、ジイリノレオイルホスファチジルコリン、ジ－トランス－２，トランス－４－オクタデカジエノイルホスファチジルコリン、ジステアロイルホスファチジルコリン、β－メチル－γ－ヘキサデシルホスファチジルコリン及びβ－オレオイル－γ－バルミトイルホスファチジルコリン等）、ホスファチジルエタノールアミン（ウシ脳由来のＩ型ホスファチジルエタノールアミン、大腸菌由来のＶ型ホスファチジルエタノールアミン、鶏卵黄身由来のホスファチジルエタノールアミン、大豆由来のホスファチジルエタノールアミン及びジミリストイルホスファチジルエタノールアミン等）、ホスファチジルグリセロール（ホスファチジルグリセロールナトリウム、ホスファチジルグリセロール、ジミリストイルホスファチジルエタノールアミンナトリウム及びウシ心臓由来のカルジオリピン２ナトリウム等）、ホスファチジルセリン（ジパルミトイルホスファチジルセリン、ジパルミトイルホスファチジルセリンナトリウム及びウシ脳由来のホスファチジルセリンナトリウム等）、ホスファチジルイノシトール（小麦由来のホスファチジルイノシトールナトリウム等）及びスフィンゴミエリン｝等が挙げられる。

【００８４】リゾフォスファチジル基（Ａ５）を有する化合物としては、上記の（Ａ５）を有するモノマーからなる（共）重合体のほか、リゾフォスファチジル基含有化合物｛リゾホスファチジン酸（オレイルリゾホスファチジン酸ナトリウム及びステアリルリゾホスファチジン酸等）、リゾホスファチジルコリン（鶏卵黄身由来のリゾホスファチジルコリン、デカノイルリゾホスファチジルコリン及びステアロイルリゾホスファチジルコリン等）、リゾホスファチジルセリン（ウシ脳由来のリゾホスファチジルセリン２ナトリウム等）及びリゾホスファチジルイノシトール（大豆由来のリゾホスファチジルイノシトールナトリウム等）等｝等が挙げられる。

【００８５】糖（Ｂ１）を有する化合物としては、上記の（Ｂ１）を有するモノマーからなる（共）重合体のほか、糖含有化合物｛糖脂質（アシアロガングリオシド、ガングリオシド、シアリルルイス、シアリルラクトテトラオシルセラミド、シアリルネオラクトテトラオシルセラミド、ガラクロシルセラミド、グロボテトラシルセラミド、グルコセレブロシド、グルコシルセラミ及びラクロシルセラミド等）、単糖類（ガラクトース、グルコース、マンノース、フコース、グルコサミン、ガラクトサミン、Ｎ-アセチルガラクトサミン、Ｎ-メチルグルコサミン、Ｎ-アセチルニューラミン酸、アラビノース、デオキシグルコース、デオキシフルオログルコース、リボース、デオキシリボース、エリスロース、フルクトース、イノシトール、リクソース、マドゥロース、ムラミン酸、デオキシマンオース、ソルボース、キシロース及びトリアセチルグルカール等）、多糖類（セロビオース、ジフルクトース、コジビオース、ラクトース、ラクツロース、マルチトール、マルトース、トレハロース、ガラクトシラクトース、グルコシルスクロース、イソマルトオリゴサッカライド、マルトオリゴサッカライド、テハロサミン、パノース、キシロオリゴサッカロースフフルクトオリゴサッカライド、ニストース、スタキロース、キトオリゴ糖、キトサンオリゴ糖、ムコ多糖、アルギニン酸、カードラン、デキストラン、レバン、パラミオール、ポリデキストロース、プルラン、スターチ及びシクロデキストリン等）｝等が挙げられる。

【００８６】ステロイド環（Ｂ２）を有する化合物としては、上記の（Ｂ２）を有するモノマーからなる（共）重合体のほか、ステロイド環含有化合物｛天然又は合成の胆汁酸（ケノジオール、コール酸、デオキシコール酸、グリココール酸、グリコリトコール酸、リソコール酸、スクアレン及びタウロコール酸等）及び天然又は合成のステロール（ブラシカステロール、カンペステロール、コレステロール、エルゴステロール、フコステロール、ラノステロール、シトステロール及びスチグマステ

ロール等）等｝等が挙げられる。

【００８７】基材にこれらの化合物を接触させる方法としては、前記、ポリペプチド（Ｐ）を基材に接触させる方法と同様の方法が適用できる。これらの化合物の使用量（ｇ／ｃｍ²）は、基材の表面積１ｃｍ²当り、１×１０⁻⁸以上が好ましく、さらに好ましくは１×１０⁻⁶以上である。また1以下が好ましく、さらに好ましくは１×１０⁻²以下である。

【００８８】④の方法において、官能基（Ａ）及び／又は構造（Ｂ）を有する化合物としては、③の方法で用いることができる化合物と同じものが使用できる。官能基（Ａ）及び／又は構造（Ｂ）を有する化合物を基材中に混合する方法としては、基材を構成する素材と（Ａ）及び／又は（Ｂ）を有する化合物とを混合した後に成型する方法や、基材がビニル樹脂の場合、（Ａ）及び／又は（Ｂ）を有する化合物の存在下にビニル樹脂を構成するモノマーを公知の方法で重合させる方法等が挙げられる。これら化合物の使用量（ｇ／ｃｍ²）は、基材の表面積１ｃｍ²当り、１×１０⁻⁸以上が好ましく、さらに好ましくは１×１０⁻⁶以上である。また1以下が好ましく、さらに好ましくは１×１０⁻²以下である。

【００８９】本発明のポリペプチド含有基材は、細胞培養用に好適に使用されるほか、火傷などの治療に用いられる創傷被覆剤や、体内埋め込み型の組織再生用材料として使用できるものである。本発明のポリペプチド含有基材を用いて、培養される動物細胞の種類としては特に制限がなく、ヒト、サル、マウス、ハムスター、ラット、イヌ及び昆虫等の初代培養細胞や株化細胞、並びに細胞培養実験、医薬品又はワクチン生産等に用いられる公知の細胞等が使用できる。

【００９０】具体的には例えば、Ｖｅｒｏ（アフリカミドリザル腎）細胞、ＣＨＯ（チャイニーズハムスター卵巣）細胞、ＭＤＣＫ（イヌ腎）細胞、ＷＩ３８（ヒト胎児肺）細胞、ヒト由来の幹細胞、内皮細胞、上皮細胞、実質細胞、線維芽細胞及び角質細胞等の正常細胞等が挙げられる。これら細胞の中では、無血清培養のニーズが高く、本発明のポリペプチド含有基材の特徴が最大限活用される医薬品やワクチン生産用に用いられる細胞（Ｖｅｒｏ細胞、ＭＤＣＫ細胞、ＣＨＯ細胞及び正常細胞等）に最適である。

【００９１】本発明の基材を用いて動物細胞を培養する方法としては特に制限はなく、通常の方法、例えば、朝倉書店発行日本組織培養学会編「組織培養の技術」に記載されている方法等が適用できる。例えば、基材としてＴ－フラスコ（培養面積；１０～５００ｃｍ²等）、プレート（２～３８４穴等）又はディッシュ（培養面積；５～５００ｃｍ²等）を用いる場合、未成熟肥満細胞を５０～５００万個／ｍＬの濃度で分散した培地を、深さが１～２０ｍｍになる量だけディッシュ又はシャーレに加え、ＣＯ₂濃度５体積%、３７℃の炭酸ガスインキュベーター中で静置培養する方法等が挙げられる。この際、１～５日毎に、１／３量～全量の培地を交換することが好ましい。

【００９２】基材としてローラーボトル（容量；０．１～１０Ｌ等）を用いる場合、未成熟肥満細胞を５０～５００万個／ｍＬの濃度で分散した培地を、０．５／１００～３０／１００の容量になる量だけローラーボトルに加え、ＣＯ₂濃度５体積%、３７℃の炭酸ガスインキュベーター中で、０．１～１０ｒｐｍの回転速度で回転させながら培養する方法等が挙げられる。この際、１２～５日毎に、１／３量～全量の培地を交換することが好ましい。

【００９３】基材としてマイクロキャリアビーズ（粒子径；２０～５００μｍ、密度；１．０～１．１ｇ／ｃｍ³、表面積；１００～１００，０００ｃｍ²／ｇ等）を用いる場合、スピナーフラスコ（容量；１０～５００ｍＬ等）中に、未成熟肥満細胞を５０～５００万個／ｍＬの濃度で分散した培地を、１／１０～７／１０の容量になる量だけ加え、マイクロキャリアビーズを、培地１Ｌ当り０．１～５０ｇの割合で加え、ＣＯ₂濃度５体積%、３７℃の炭酸ガスインキュベーター中で、１～１００ｒｐｍの回転速度で撹拌しながら培養する方法等が挙げられる。この際、１２～５日毎に、１／３量～全量の培地を交換することが好ましい。さらにスピナーフラスコの代わりに流動層型バイオリアクターや充填型バイオリアクター（容量；１０ｍＬ～１０ｋＬ等）を用いて、マイクロキャリアビーズをバイオリアクター内にセットし未成熟肥満細胞を５０～５００万個／ｍＬの濃度で分散した１～５倍容量の培地を循環させた後、ＣＯ₂濃度５体積%、３７℃の炭酸ガスインキュベーター中で調整した培地を１～１００ｃｍ／分の線速度で循環しながら連続培養する方法等が挙げられる。

【００９４】基材としてホローファイバー（内径１０～５００μｍ等）を用いる場合、カートリッジ（容量；１０～１０００ｍＬ等）中に、未成熟肥満細胞を５０～５００万個／ｍＬの濃度で分散した培地をカートリッジに加えたあと、ホローファイバー内に、ＣＯ₂濃度５体積%、３７℃の炭酸ガスインキュベーター中で調整した培地を１～１００ｃｍ／分の線速度で循環し連続培養する方法等が挙げられる。

【００９５】培養後は、ＥＤＴＡ等のキレート剤若しくはトリプシン等の蛋白質分解酵素で処理するか又はスクレーパーで掻きとることによって、成熟肥満細胞が回収される。これらのうち、ＥＤＴＡで処理する方法が好ましい。

【００９６】培地としては、用いる動物細胞の種類に応じて、ＭＥＭ培地、ＢＭＥ培地、ＤＭＥ培地、αＭＥＭ培地、ＩＭＥＭ培地、ＥＳ培地、ＤＭ－１６０培地、Ｆｉｓｈｅｒ培地、Ｆ１２培地、ＷＥ培地及びＲＰＭＩ培地等、朝倉書店発行「日本組織培養学会編 組織培養の

技術第三版」５８１頁に記載の基礎培地、これらの培地に血清成分（ウシ胎児血清等）等を添加したもの、並びに市販の無血清培地［味の素（株）製無血清培地ＡＳＦ１０３，同ＡＳＦ１０４，同ＡＳＦ３０１、ギブコ社製無血清培地ＣＨＯ－ＳＦＭ，同ＶＰ－ＳＦＭ等］等が用いられる。

【００９７】血清培地を使用した場合、血清中に成分未知の蛋白質等が含まれ再現性が得られにくいこと、細胞を用いる医薬品生産の場合には精製工程が複雑となりコストがかかること、さらにウィルス感染の危険性があること等の理由から、血清を含まないいわゆる無血清培地が好ましく、特に、本発明の基材は、無血清培地でも細胞の接着・増殖性に優れているため、本発明の基材とともに用いる培地としては無血清培地が特に好ましい。

【００９８】さらに必要に応じて、細胞増殖因子（Ｓ）を培地中に含有させることにより、動物細胞の増殖速度をさらに高めたり、細胞活性を高めたり、細胞が本来有する機能を発現させたりすることができる。細胞増殖因子（Ｓ）は細胞を増殖させる活性のある物質であり、例えば、ＦＧＦ、ＶＥＧＦ、ＨＧＦ、ＥＧＦ、ＰＤＧＦ、ＩＧＦ及びＢＭＰ等が挙げられ、この他に、例えば財団法人名古屋大学出版会発行「上田実編ティッシュエンジニアリング（１９９９年）」４３～５１頁及び同文献に付記されている参考文献に記載されているもの等も用いられる。

【００９９】

【実施例】以下に実施例を掲げて本発明を更に詳しく説明するが、本発明はこれら実施例のみに限定されるものではない。

＜実施例１＞スチレン９９重量部、ジビニルベンゼン１重量部を懸濁重合して得られたポリスチレンビーズを金属製網篩（ＪＩＳ　Ｚ８８０１－２０００）で篩い分けして、７５～１０６μｍの間の粒子径を有するビーズを得た。レーザー式粒度分布測定装置ＬＡ－９２０（堀場製作所製）で測定した体積平均粒径は、９６μｍであった。このビーズの平均表面積は、５９５ｃｍ²／ｇと計算された。

【０１００】特表平３－５０２９３５号公報中の実施例記載の方法に準じて、（Gly Ala Gly Ala Gly Ser）₃配列（１０）とArg Gly Asp配列との繰り返し単位からなるポリペプチドを、遺伝子組み替え大腸菌を増殖させ、破砕し、抽出することにより作成し、（Ｍｎ）の違いによって精製し、以下のポリペプチドを単離した。

【０１０１】ポリペプチド（Ｆ１）；（Ｍｎ）８０，０００、１分子あたりのＲＧＤ配列の数１０個、（ＧＡＧＡＧＳ）₃配列の数１０個。

ポリペプチド（Ｆ２）；（Ｍｎ）２４０，０００、１分子あたりのＲＧＤ配列の数３０個、（ＧＡＧＡＧＳ）₃配列の数３０個。

ポリペプチド（Ｆ３）；（Ｍｎ）５０，０００、１分子あたりのＲＧＤ配列の数６個、（ＧＡＧＡＧＳ）₃配列の数６個。

ポリペプチド（Ｆ４）；（Ｍｎ）５００，０００、１分子あたりのＲＧＤ配列の数６０個、（ＧＡＧＡＧＳ）₃配列の数６０個。

【０１０２】次いで、ポリペプチド（Ｆ１）の４．５規定の過塩素酸リチウム溶液（ポリペプチドの濃度；１ｍｇ／ｍｌ）をリン酸バッファー液（ＰＢＳ）でポリペプチド（Ｆ１）の濃度が１００μｇ／ｍｌとなるように希釈し、ポリペプチド（Ｆ１）溶液（１）を作製した。

【０１０３】１０ｍｌ試験管にジメチルアミノエチルメタクリレート２ｇ、アゾビスイソヴァレロニトリル０．０４ｇおよびジオキサン２ｇを加え、窒素置換後、密閉下、７０℃湯浴中で４時間振盪した。得られた溶液をヘキサン１００ｍＬ中に滴下し、析出したポリマー分を回収し乾燥することによりジメチルアミノエチルメタクリレート重合体（ＰＤＡＭ）を得た。次いで、ＰＤＡＭをイオン交換水に溶解し、ＰＤＡＭの濃度が１００μｇ／ｍｌのＰＤＡＭ溶液を作製した。

【０１０４】ポリペプチド溶液（１）３７ｍｌ及びＰＤＡＭ溶液３７ｍｌの混合物に上記で得られたビーズ５ｇを加え、ポリフッ化エチレン製撹拌子で１２時間、２０～３０℃の温度下で撹拌した。得られたビーズスラリーを振盪器上にセットしたステンレス製バットに移し、１００℃の熱風を吹きつけながら、２４時間振盪乾燥した。得られた乾燥ビーズをＰＢＳ５０ｍｌで２回洗浄し、ＰＢＳ中で１２１℃で２０分間オートクレーブ滅菌後、ＵＶ照射下に乾燥することにより、本発明のポリペプチド含有基材１を得た。

【０１０５】ポリペプチド含有基材１を４．５規定の過塩素酸リチウム溶液中で２４時間、３７℃で撹拌後、過塩素酸リチウム溶液上清中に溶出したポリペプチド（Ｆ１）の含有量をピアスケミカル社製ＢＣＡ蛋白試薬で測定することによって、ポリペプチド含有基材１のポリペプチド（Ｆ１）含有量を求めた結果、１．１μｇ／ｃｍ²であった。

【０１０６】ポリペプチド含有基材１１ｇをイオン交換水５０ｍｌに分散し、を１／１００規定硝酸銀水溶液で電位差滴定することによって、ポリペプチド含有基材１の表面に存在するジメチルアミノ基の量を求めた結果、５×１０¹⁵個／ｃｍ²であった。

【０１０７】＜実施例２＞ＰＤＡＭ溶液の代わりに、ジシアンジアミド・ジエチレントリアミン・尿素重縮合物（商品名：サンフィックス４１４、三洋化成工業株式会社製）を用い、固形分を１００μｇ／ｍｌ・溶液とする以外は実施例１と同様にして、本発明のポリペプチド含有基材２を得た。実施例１と同様にして求めたポリペプチド含有基材２のポリペプチド（Ｆ１）含有量は１．１μｇ／ｃｍ²であり、また１／１００規定塩酸溶液で電位差滴定して得られた２級及び３級アミンの量は１×１

0^{16}個／ｃｍ²であった。

【０１０８】＜実施例３＞ＰＤＡＭ溶液の代わりに、ジパルミトイルホスファチジルグリセロールの１００μｇ／ｍｌ水溶液を用いる以外は実施例１と同様にして、本発明のポリペプチド含有基材３を得た。実施例１と同様にして求めたポリペプチド含有基材３のポリペプチド（Ｆ１）含有量は１．１μｇ／ｃｍ²であり、また、カイノス社製リン脂質測定キット「アクアオート　カイノス　ＰＬ試薬」を用いて測定したホスファチジル基の量は、1×10^{16}個／ｃｍ²であった。

【０１０９】＜実施例４＞ＰＤＡＭ溶液の代わりに、コール酸の１００μｇ／ｍｌ水溶液を用いる以外は実施例１と同様にして、本発明のポリペプチド含有基材４を得た。実施例１と同様にして求めたポリペプチド含有基材４のポリペプチド（Ｆ１）含有量は１．１μｇ／ｃｍ²であり、また、ロッシュダイアグノスティックス社製Ｆ－キット（ステロイド用）を用いて測定したステロイド環の量は、4×10^{16}個／ｃｍ²であった。

【０１１０】＜実施例５＞ポリペプチド（Ｆ１）の代わりに、ポリペプチド（Ｆ２）を用いる以外は実施例１と同様にして本発明のポリペプチド含有基材５を得た。実施例１と同様にして求めたポリペプチド含有基材５のポリペプチド（Ｆ２）含有量は１．０μｇ／ｃｍ²であり、また、１／１００規定硝酸銀水溶液で電位差滴定することによって、測定されたジメチルアミノ基の量は、2×10^{15}個／ｃｍ²であった。

【０１１１】＜実施例６＞ポリペプチド（Ｆ１）の代わりに、ポリペプチド（Ｆ３）を用いる以外は実施例１と同様にして本発明のポリペプチド含有基材６を得た。実施例１と同様にして求めたポリペプチド含有基材６のポリペプチド（Ｆ３）含有量は０．９μｇ／ｃｍ²であり、また、１／１００規定硝酸銀水溶液で電位差滴定することによって、測定されたジメチルアミノ基の量は、2×10^{15}個／ｃｍ²であった。

【０１１２】＜実施例７＞ポリペプチド（Ｆ１）の代わりに、ポリペプチド（Ｆ４）を用いる以外は実施例１と同様にして本発明のポリペプチド含有基材７を得た。実施例１と同様にして求めたポリペプチド含有基材７のポリペプチド（Ｆ４）含有量は１．２μｇ／ｃｍ²であり、また、１／１００規定硝酸銀水溶液で電位差滴定することによって、測定されたジメチルアミノ基の量は、4×10^{15}個／ｃｍ²であった。

【０１１３】＜実施例８＞特表平３－５０２９３５号公報中の実施例記載の方法に準じて、（Gly AlaGly Ala Gly Ser）₉配列（１０）とIle Lys Val Ala Val配列（７）とを７個含む（Ｍｎ）約９万の遺伝子組換え大腸菌の産生蛋白質ポリペプチドＬを作成した。ポリペプチド（Ｆ１）の代わりに、このポリペプチドＬを用いる以外は実施例１と同様にして本発明のポリペプチド含有基材８を得た。実施例１と同様にして求めたポリペプチド含有基材８のポリペプチドＬの含有量は１．１μｇ／ｃｍ²であり、また、１／１００規定硝酸銀水溶液で電位差滴定することによって、測定されたジメチルアミノ基の量は、3×10^{15}個／ｃｍ²であった。

【０１１４】＜実施例９＞ＰＤＡＭの代わりに、和光純薬（株）製ポリエチレンイミン（分子量＝７０，０００）を用いる以外は実施例１と同様にして、本発明のポリペプチド含有基材９を得た。実施例１と同様にして求めたポリペプチド含有基材９のポリペプチド（Ｆ１）含有量は１．１μｇ／ｃｍ²であり、また１／１００規定塩酸溶液で電位差滴定して得られた２級アミンの量は4×10^{16}個／ｃｍ²であった。

【０１１５】＜実施例１０＞ＰＤＡＭ溶液の代わりに、ＰＤＡＭ溶液１００ｍＬにメチルクロリド５ｇを加え、８０℃で８時間反応させて得られた溶液を用いる以外は実施例１と同様にして、本発明のポリペプチド含有基材１０を得た。実施例１と同様にして求めたポリペプチド含有基材１０のポリペプチド（Ｆ１）含有量は１．１μｇ／ｃｍ²であり、また１／１００規定塩酸溶液で電位差滴定して得られた４級アンモニウム塩の量は2×10^{16}個／ｃｍ²であった。

【０１１６】＜実施例１１＞ＰＤＡＭの代わりに、和光純薬工業（株）製ガングリオシドＧ　ボビンブレインを用いる以外は実施例１と同様にして、本発明のポリペプチド含有基材１１を得た。実施例１と同様にして求めたポリペプチド含有基材１１のポリペプチド（Ｆ１）含有量は１．１μｇ／ｃｍ²であり、また、ロッシュダイアグノスティックス社製「Ｆ－キット　糖質用」を用いて得られた糖の量は1×10^{16}個／ｃｍ²であった。

【０１１７】＜実施例１２＞ポリペプチド溶液（１）の代わりに、ポリペプチド（Ｆ１）の濃度が４５μｇ／ｍｌのポリペプチド溶液（２）を用いる以外は実施例１と同様にして、本発明のポリペプチド含有基材１２を得た。実施例１と同様にして求めたポリペプチド含有基材１２のポリペプチド（Ｆ１）含有量は０．３μｇ／ｃｍ²であり、また、１／１００規定硝酸銀水溶液で電位差滴定することによって、測定されたジメチルアミノ基の量は、2×10^{15}個／ｃｍ²であった。

【０１１８】＜実施例１３＞ポリペプチド溶液（１）の代わりに、ポリペプチド（Ｆ１）の濃度が１０００μｇ／ｍｌのポリペプチド溶液（３）を用いる以外は実施例１と同様にして、本発明のポリペプチド含有基材１３を得た。実施例１と同様にして求めたポリペプチド含有基材１３のポリペプチド（Ｆ１）含有量は１０．０μｇ／ｃｍ²であり、また、Ｎ／１００硝酸銀水溶液で電位差滴定することによって、測定されたジメチルアミノ基の量は、3×10^{15}個／ｃｍ²であった。

【０１１９】＜実施例１４＞スチレン９８重量部、ジビニルベンゼン1重量部、ジメチルアミノエチルメタクリレート１重量部を懸濁重合して得られたポリスチレンビ

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ーズを金属製網篩（ＪＩＳ　Ｚ８８０１－２０００）で篩い分けして、７５～１０６μｍの間の粒子径を有するビーズを得た。レーザー式粒度分布測定装置ＬＡ－９２０（堀場製作所製）で測定した体積平均粒径は、９３μｍであった。このビーズの平均表面積は、６２６ｃｍ²／ｇと計算された。

【０１２０】ポリペプチド溶液（１）３７ｍｌに上記で得られたビーズ５ｇを加え、ポリフッ化エチレン製撹拌子で１２時間、２０～３０℃の温度下で撹拌した。得られたビーズスラリーを振盪器上にセットしたステンレス製バットに移し、１００℃の熱風を吹きつけながら、２４時間振盪乾燥した。得られた乾燥ビーズをＰＢＳ５０ｍｌで２回洗浄し、ＰＢＳ中で１２１℃で２０分間オートクレーブ滅菌後、ＵＶ照射下に乾燥することにより、本発明のポリペプチド含有基材１４を得た。

【０１２１】実施例１と同様にして求めたポリペプチド含有基材１４のポリペプチド（Ｆ１）含有量は０．９μｇ／ｃｍ²であり、また、１／１００規定硝酸銀水溶液で電位差滴定することによって測定されたジメチルアミノ基の量は、１×１０¹⁷個／ｃｍ²であった。

【０１２２】＜比較例１＞ポリペプチド溶液（１）の代わりに、ＰＢＳを用いる以外は実施例１と同様にして、比較用基材１５を得た。実施例１と同様にして求めた比較用基材１５のジメチルアミノ基の量は４×１０¹⁵個／ｃｍ²であった。

【０１２３】＜比較例２＞ＰＤＡＭ溶液の代わりに、ＰＢＳを用いる以外は実施例１と同様にして、比較用基材１６を得た。実施例１と同様にして求めた比較用基材１６のＳＬＰＦ含有量は１．０μｇ／ｃｍ²であった。

【０１２４】＜比較例３＞ＰＤＡＭの代わりに、和光純薬工業（株）製ポリ－Ｌ－リジンを用いる以外は実施例１と同様にして、比較用基材１７を得た。実施例１と同様にして求めた比較用基材１７のＳＬＰＦ含有量は１．１μｇ／ｃｍ²であり、また１級アミノ基の量は１×１０¹⁴個／ｃｍ²であった。

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＊【０１２５】＜比較例４＞ポリペプチド溶液（１）の代わりに、ＰＢＳを用いる以外は実施例２と同様にして、比較用基材１８を得た。実施例２と同様にして求めた比較用基材１８の２級及び３級アミンの量は１×１０¹⁵個／ｃｍ²であった。

【０１２６】＜比較例５＞ポリペプチド溶液（１）の代わりに、ＰＢＳを用いる以外は実施例３と同様にして、比較用基材１９を得た。実施例３と同様にして求めた比較用基材１９のホスファチジル基の量は、１×１０¹⁵個／ｃｍ²であった。

【０１２７】＜比較例６＞ポリペプチド溶液（１）の代わりに、ＰＢＳを用いる以外は実施例４と同様にして、比較用基材２０を得た。実施例４と同様にして求めた比較用基材２０のステロイド環の量は、４×１０¹⁵個／ｃｍ²であった。

【０１２８】＜細胞培養＞得られた基材１～２０を用いて無血清細胞培養実験を行った。２０個の１Ｌスピナーフラスコに基材１～２０を６ｇづつそれぞれ加え、２４５ｍｌのインビトロジェン社製無血清培地ギブコＯｐｔｉＰｒｏ-ＳＦＭを加え、３７℃で２０分間撹拌下温調後、ＭＤＣＫ細胞［大日本製薬（株）から購入］を細胞濃度：５．０×１０⁵個／ｍＬに分散したもの５ｍｌを加え（細胞密度＝１０万個／ｍｌ）、２５ｒｐｍで撹拌しながら、二酸化炭素と空気の混合物（二酸化炭素／空気＝５／９５体積比）中、３７℃で培養を行なった。基材への初期接着性を評価するため３０分後に、また、増殖性を評価するため１日後及び３日後に、撹拌下に基材の懸濁液１ｍｌをそれぞれサンプリングし、ＰＢＳで基材を洗浄して基材に接着していない細胞を除去した後、細胞核数をウィーゼル（Wezel）によるクリスタルバイオレットを用いた細胞核計数法（nuclei-counting method）で計数することで、細胞密度（単位：万個/ｍｌ）を測定した。結果を表１に示す。

【０１２９】

＊【表１】

	実施例														比較例					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6
基材番号	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
３０分後	5	6	4	6	4	4	6	5	6	4	6	5	5	4	3	2	4	3	1	2
１日後	18	19	18	19	18	17	20	18	18	17	19	18	18	17	5	9	12	6	8	7
３日後	75	80	70	78	75	70	78	75	80	70	78	77	72	74	15	40	50	22	32	34

【０１３０】なお、比較例１の基材１５を用い、インビトロジェン社製無血清培地ギブコＯｐｔｉＰｒｏ-ＳＦＭの代わりに、血清培地（ＦＢＳ１０%含有ＭＥＭＥ）を用いた場合の細胞密度（万個／ｍｌ）は、３０分後；５、１日後；１６、３日後；７０であった。

【０１３１】＜実施例１５＞旭テクノグラス製６０ｍｍφ細胞培養ディッシュ（培養面積；２８ｃｍ²／個）に、実施例１で得られたポリペプチド溶液（１）とＰＤＡＭ溶液の当重量混合物をＰＢＳで１０倍に希釈したものを1．５ｍｌ加え、２０～２５℃のクリーンベンチ内でで１週間放置することによって、自然乾燥させた。さらにＰＢＳ５ｍｌで２回洗浄して、本発明のポリペプチド含有基材（ディッシュ１）を得た。

【０１３２】ディッシュ１に４．５規定の過塩素酸リチウム溶液３ｍｌを加え、３７℃で４８時間振盪後、過塩素酸リチウム溶液上清中に溶出したポリペプチド（Ｆ１）の含有量をピアスケミカル社製ＢＣＡ蛋白試薬で測定することによって、ディッシュ１のポリペプチド（Ｆ

１）含有量を求めた結果、２．０μｇ／ｃｍ²であった。ディッシュ１にイオン交換水１．５ｍｌ加え、１／１００規定硝酸銀水溶液で電位差滴定することによって、ディッシュ１の表面に存在するジメチルアミノ基の量を求めた結果、１×１０¹⁷個／ｃｍ²であった。

【０１３３】＜実施例１６＞ポリペプチド（Ｆ１）の代わりにポリペプチドＬを用いる以外は実施例１５と同様にして、本発明のポリペプチド含有基材（ディッシュ２）を得た。実施例１５と同様にして求めたディッシュ２のポリペプチドＬ含有量は２．１μｇ／ｃｍ²であり、また、１／１００規定硝酸銀水溶液で電位差滴定することによって、測定されたジメチルアミノ基の量は、１×１０¹⁷個／ｃｍ²であった。

【０１３４】＜比較例７＞ポリペプチド溶液（１）の代わりに、ＰＢＳを用いる以外は実施例１５と同様にして、比較用のディッシュ３を得た。実施例１５と同様にして求めたディッシュ３のジメチルアミノ基の量は４×１０¹⁶個／ｃｍ²であった。

【０１３５】＜細胞培養＞得られたディッシュ１～３を用いて無血清細胞培養実験を行った。各ディッシュに、インビトロジェン社製無血清培地ギブコＯｐｔｉＰｒｏ－ＳＦＭに、ＭＤＣＫ細胞［大日本製薬（株）から購入］を細胞濃度：２万個／ｍＬに分散したもの５ｍｌを加え（総細胞数；１０万個）、二酸化炭素と空気の混合物（二酸化炭素／空気＝５／９５体積比）中、３７℃で３日間培養を行なった。ディッシュをトリプシンで処理＊し細胞を浮遊させ、血球計数盤を用いて顕微鏡で細胞数（万個）を測定した。その結果を表２に示す。

【０１３６】

【表２】

	実施例		比較例
	１５	１６	７
ディッシュ番号	１	２	３
細胞数（万個）	７２	７８	４２

【０１３７】なお、比較例７のディッシュ３を用いて、インビトロジェン社製無血清培地ギブコＯｐｔｉＰｒｏ－ＳＦＭの代わりに、血清培地（ＦＢＳ１０％含有ＭＥＭＥ）を用いた場合の細胞数は、６５万個であった。

【０１３８】以上の実施例及び比較例から、本発明のポリペプチド含有基材を用いた場合、無血清培養においても血清培地を使用した場合と同様以上かつ、ポリペプチド（Ｆ１）等単独をコーティングしたものよりも優れた細胞接着性及び細胞増殖性が得られ、細胞を効率的に培養できることが判る。

【０１３９】

【発明の効果】本発明の基材を用いると、無血清でも細胞を効率的に培養が可能であり、動物細胞を用いる医薬品やワクチンの生産の完全無血清化が実現できるものである。

【０１４０】

【配列表】

<110>三洋化成工業株式会社；SANYO CHEMICAL INDUSTRIES,LTD.
<120>ポリペプチド含有基材
<160>11
<210>1
<211>4
<212>PRT
<213>Homo sapiens
<400>1
Arg Glu Asp Val
1
<210>2
<211>5
<213>PRT
<213>Homo sapiens
<400>2
Tyr Ile Gly Ser Arg
1 5
<210>3
<211>5
<213>PRT
<213>Homo sapiens
<400>3
Pro Asp Ser Gly Arg
1 5

<210>4
<211>7
<213>PRT
<213>Homo sapiens
<400>4
Arg Tyr Val Val Leu Pro Arg
1 5

<210>5
<211>6
<213>PRT
<213>Homo sapiens
<400>5
Leu Gly Thr Ile Pro Gly
1 5
<210>6
<211>10
<213>PRT
<213>Homo sapiens
<400>6
Arg Asn Ile Ala Glu Ile Ile Lys Asp Ile
1 5 10
<210>7
<211>5
<213>PRT
<213>Homo sapiens
<400>7
Ile Lys Val Ala Val
1 5
<210>8
<211>4
<213>PRT
<213>Homo sapiens
<400>8
Asp Gly Glu Ala
1
<210>9
<211>6
<213>PRT
<213>Bombyx mori
<400>9
Gly Ala Gly Ala Gly Ser
1 5
<210>10
<211>54
<213>PRT
<213>Bombyx mori
<400>10
Gly Ala Gly Ala Gly Ser Gly Ala Gly Ala Gly Ser Gly Ala Gly Ala
1 5 10 15

31 32

Gly Ser Gly Ala Gly Ala Gly Ser Gly Ala Gly Ala Gly Ser Gly Ala
20 25 30
Gly Ala Gly Ser Gly Ala Gly Ala Gly Ser Gly Ala Gly Ala Gly Ser
35 40 45
Gly Ala Gly Ala Gly Ser
50

<210>11
<211>30
<213>PRT
<213>Homo sapiens
<400>11

Gly Ala Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly
1 5 10 15
Ala Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro Gly Pro Pro
20 25 30